Translational Immunology Program, Research Program Unit

University of Helsinki

Department of Pathology

University of Helsinki

Helsinki University Hospital

Neurology, Neurocenter

University of Helsinki

Helsinki University Hospital

Finland

Genetics of neurodegeneration: phenotypic effects of C9orf72 intermediate-length alleles and the association of genetic and neuropathological features of dementia

Karri Kaivola

Academic dissertation

To be publicly discussed, with the permission of the Faculty of Medicine of the University of Helsinki, 15.1.2021 at 1pm, Porthania, lecture hall P674, Yliopistonkatu 3, Helsinki

Helsinki 2020

Supervisors

Professor Pentti Tienari
Translational Immunology Program, Research Program Unit
University of Helsinki and Helsinki University Hospital

Adjunct Professor Liisa Myllykangas
Department of Pathology
University of Helsinki and HUSLAB, Helsinki University Hospital

Reviewers

Adjunct Professor Mikko Kärppä
Medical Research Center Oulu
Oulu University Hospital and University of Oulu

Adjunct Professor Annakaisa Haapasalo
A.I. Virtanen Institute for Molecular Sciences
University of Eastern Finland

Opponent

Professor Anne Remes
Unit of Clinical Neuroscience, Neurology and Medical Research Center Oulu
University of Oulu and Oulu University Hospital

ISBN 978-951-51-6877-1 (pbk.)
ISBN 978-951-51-6878-8 (PDF)

Unigrafia
Helsinki 2020

To all who made this possible

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. Kaivola K, Kiviharju A, Jansson L, Rantalainen V, Eriksson JG, Strandberg TE, Laaksovirta H, Renton AE, Traynor BJ, Myllykangas L, Tienari PJ. *C9orf72* Hexanucleotide Repeat Length in Older Population: Normal Variation and Effects on Cognition. Neurobiol Aging. 2019 Dec;84:242.e7-242.e12.

II. Kaivola K, Salmi SJ, Jansson L, Launes J, Hokkanen L, Niemi AK, Majamaa K, Lahti J, Eriksson JG, Strandberg T, Laaksovirta H, Tienari PJ. Carriership of two copies of *C9orf72* hexanucleotide repeat intermediate-length alleles is a risk factor for ALS in the Finnish population. Acta Neuropathol Commun. 2020 Nov 9;8(1):187.

III. Raunio A, Kaivola K, Tuimala J, Kero M, Oinas M, Polvikoski T, Paetau A, Tienari PJ, Myllykangas M. Lewy-related Pathology Exhibits Two Anatomically and Genetically Distinct Progression Patterns: A Population-Based Study of Finns Aged 85. Acta Neuropathologica. 2019 November; 138 (5), 771-782

IV. Mäkelä M*, Kaivola K*, Valori M, Paetau A, Polvikoski T, Singleton AB, Traynor BJ, Stone DJ, Peuralinna T, Tienari PJ, Tanskanen M, Myllykangas L. Alzheimer risk loci and associated neuropathology in a population-based study (Vantaa 85+). Neurol Genet. 2018 Jan 18;4(1):e211

* equal contribution.

The article “Alzheimer risk loci and associated neuropathology in a population-based study (Vantaa 85+)” was also included in Mira Mäkelä’s thesis ”Neuropathological and genetic determinants of dementia: a prospective and population-based study of very elderly Finns” (ISBN 978-951-51-4472-0).

ABSTRACT

In neurodegenerative diseases neurons gradually die leading to various symptoms based on the affected nervous region. Often memory, movements or both are affected, and the symptoms worsen over time greatly affecting the quality of life. Neurodegenerative diseases are often caused by many factors, both intrinsic and environmental. Genetics often play an important role in neurodegenerative diseases and genetic research can help to elucidate disease mechanisms. Since many neurodegenerative diseases share overlapping mechanisms, insights in one disease can elucidate the mechanisms of other diseases as well.

The aim of this thesis was to study the genetics of three neurodegenerative diseases: amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and the closely related dementia with Lewy bodies (DLB). ALS is characterized by a progressive loss of voluntary movements that leads to respiratory failure. AD and DLB lead to dementia but DLB patients often also have parkinsonism and visual hallucinations.

In Publication I, we determined the *C9orf72* GGGGCC hexanucleotide repeat length of 3142 Finns. The *C9orf72* hexanucleotide expansion is the most common cause of ALS and frontotemporal lobar degeneration (FTLD) in populations of European ancestry, but the exact pathogenic repeat length and the role of intermediate length alleles in disease is unclear. Often 30 repeats is used as the expansion threshold. We presented the distribution of *C9orf72* repeat length in older individuals and found that 0.38% have 30-45 repeats, which is often considered to be a pathogenic length. However, these individuals had no apparent accumulation of neurodegenerative or psychiatric symptoms. We found no association with intermediate repeat length alleles (7-45 or 20-45) and AD or cognitive impairment. Intermediate-length alleles with ≥20 repeats were found to be more common in Finland than elsewhere.

In Publication II, we utilized the same cohorts from the first study as controls and additionally determined the repeat lengths of 750 Finnish ALS patients and additional 800 younger controls aged 18-65 years. There have been mixed results on the association of intermediate repeat length alleles with ALS so we tested the association using several thresholds: 7-45, 17-45, 21-45, 24-45 and 24-30. None of these intermediate repeats associated with ALS when only the effect of the longer allele was considered. However, carrying two copies of intermediate-length alleles was associated with ALS especially when the longer allele was over 17 repeats (p=0.002, OR 5.32, 95% CI 2.02-14.05).

In Publication III, we studied the distribution of Lewy-related pathology (LRP) in the Vantaa 85+ population cohort. Our results confirmed that LRP progresses caudo-rostrally in 64% individuals with LRP, whereas a third have amygdala-based progression pattern. Moreover, the amygdala-based progression pattern was associated with AD pathology and *APOE ε4*. These findings are important since *APOE* ε4 has been the strongest signal in association studies of DLB and it has been unclear whether *APOE* directly affects the progression of LRP

or if the effect is mediated by AD co-pathology. Our findings suggest DLB should not be viewed as a single entity, but two.

In Publication IV, we studied the association of previously identified genetic risk loci for AD with the different neuropathological features of AD. These features were amyloid β deposition (CERAD score), tau pathology (Braak staging), cerebral amyloid angiopathy and capillary amyloid β. We identified risk loci for every neuropathological feature including capillary amyloid β for which there were no previously identified risk loci. Our findings help to match known AD loci to neuropathological changes elucidating the role of each gene in AD pathogenesis.

The results of this work 1. improve the knowledge of the pathogenic repeat length of the *C9orf72* expansion, which improves diagnostics and the understanding of ALS disease mechanisms, 2. show the high prevalence of the amygdala-based LRP progression pattern and the association of *APOE* ε4 and AD pathology with it, demonstrating two major subgroups in DLB that need to be considered in the diagnostics and treatment of DLB patients, and 3. demonstrates how AD genetic risk loci associate with AD neuropathological changes including new risk loci for capillary amyloid β.

TIIVISTELMÄ (FINNISH ABSTRACT)

Hermorappeumasairauksissa hermosolut vaurioituvat ja vähitellen kuolevat. Oirekuva riippuu hermoston vauriokohdista ja rappeutumisprosessin etenemisasteesta. Loppuvaiheessa hermorappeumasairaudet vaikuttavat usein merkittävästi sairastuneen toimintakykyyn, elämänlaatuun ja minuuteen. Hermorappeumasairaudet ovat yleensä monen tekijän summa: niiden syntyyn vaikuttavat perimä ja ympäristötekijät, mutta sairauksien tarkat syntymekanismit ovat puutteellisesti tunnettuja, ja hoitokeinoja on vielä vähän. On kuitenkin havaittu, että eri hermorappeumasairauksissa on samoja syntymekanismeja ja toisinaan myös samoja perimän muutoksia. Näin ollen eri hermorappeumasairauksien tutkimuksissa tehdyt havainnot voivat avata uusia tutkimussuuntia ja hoitomahdollisuuksia laajemminkin. Perimän muutosten tutkimiseen käytetty teknologia on kehittynyt valtavasti 2000-luvulla ja sen myötä hermorappeumasairauksien riski- ja taustatekijöistä on saatu paljon arvokasta tietoa ja on löydetty jo muutamia hoitokeinojakin.

Tässä väitöskirjassa tutkittiin kolmea hermorappeumasairautta ja niihin yhteydessä olevia perimän muutoksia. Amyotrofinen lateraaliskleroosi (ALS), toiselta nimeltään motoneuronitauti, on liikehermojen rappeumasairaus, joka johtaa etenevään halvaantumiseen ja kuolemaan. Alzheimerin tauti ja sitä muistuttava Lewyn kappale –tauti ovat väestötasolla kaksi yleisintä dementoivaa hermorappeumasairautta. Dementian lisäksi Lewyn kappale –tauti aiheuttaa usein myös Parkinsonin taudin kaltaisia liikehäiriöitä ja näköharhoja.

ALS:in yleisin aiheuttaja eurooppalaisessa ja erityisesti suomalaisessa väestössä on *C9orf72*-geenissä oleva GGGGCC-toistojakson monistuma (ekspansio). Valtaosalla ihmisistä GGGGCC-toistojakso toistuu peräkkäin 2-6 kertaa, mutta ekspansiossa se voi toistua satoja tai tuhansia kertoja. Tautia aiheuttava toistojaksojen vähimmäismäärä ei ole tiedossa, mutta 30 toistojaksoa pidetään usein tällaisena rajana. Myös yli seitsemän toistojakson (ns. keskipitkien eli intermediate-alleelien) on raportoitu olevan yhteydessä eri hermorappeumasairauksiin. Ensimmäisessä osatyössä määritimme 3142 ikääntyneeltä suomalaiselta GGGGCC-toistojaksojen määrän. Tulostemme perusteella 0.38%:lla oli 30-45 toistojaksoa, vaikka heillä ei ollut viitteitä ALS:ista tai poikkeuksellisen paljon muitakaan hermorappeumasairauksia. Tämän perusteella normaalivariaation yläraja on suomalaisilla 30 sijasta vähintään 45 toistojaksoa, mikä on otettava huomioon määritettäessä toistojakson sairautta aiheuttavaa rajaa mm. potilasneuvonnassa. Analyysiemme perusteella 7-45 tai 20-45 toistojaksoa eivät vaikuttaneet merkittävästi Alzheimerin taudin tai muistihäiriön riskiin suomalaisessa iäkkäässä väestössä.

Toisessa osatyössä selvitimme ensimmäisen osatyön aineiston lisäksi toistojaksojen määrän noin 800 suomalaiselta alle 65-vuotiaalta verrokilta ja noin 750 suomalaiselta ALS-potilaalta. Tässä ALS-aineistossa GGGGCC-toistojakson ekspansio löytyi 26%:lta potilaista. Aiemmin on raportoitu myös 17-30 ja 24-30 toistojakson yhteydestä ALS:iin, mutta nämä havainnot on tehty väestöissä, joissa nämä toistojaksot ovat hyvin harvinaisia. Suomessa kyseiset toistojaksomäärät ovat yleisempiä ja osoitimme, että niillä ei ole yhteyttä ALS:iin, kun vain

pidemmän toistojakson vaikutus huomioidaan. Sen sijaan henkilöillä, joilla oli kaksi intermediate-alleelia, oli suurentunut riski sairastua ALS:iin etenkin jos toisessa alleelissa oli yli 17 toistojaksoa (p=0.002, OR 5.32, 95% CI 2.02-14.05).

Kolmannessa osatyössä selvitimme Lewyn kappale –taudissa esiintyvien hermoston neuropatologisten muutosten (LRP, Lewy-related pathology) yleisyyttä ja etenemistapaa yli 85-vuotiaiden suomalaisten väestöpohjaisessa Vantaa85+ aineistossa. Lewyn kappale -taudissa alfasynukleiini-proteiinia sisältäviä Lewyn kappaleita kertyy eri puolille aivoja. Parkinsonin taudissa samoja proteiinikertymiä taas on ensisijaisesti aivojen pohjaosissa. Tulosten perusteella pystyimme jakamaan LRP-muutosten etenemistavan kahdeksi eri tyypiksi. Toinen on aivojen pohjaosista ylöspäin etenevä muoto (caudo-rostral, 67% tapauksista) ja toinen on lähtöisin mantelitumakkeesta aivojen keskiosilta (amygdala-based, 32% tapauksista). Vain 1% tapauksista jäi luokittelun ulkopuolelle. Mantelitumakkeesta alkavat LRP-muutokset esiintyivät Alzheimerin taudin neuropatologisten muutosten kanssa ja liittyivät tunnettuun Alzheimerin taudin geneettiseen riskitekijään *APOE* ε4:ään. Sen sijaan aivojen pohjaosista alkavat LRP-muutokset (caudo-rostral) esiintyivät Alzheimer-patologiasta ja *APOE ε4*:stä riippumattomasti. Tulostemme perusteella Lewyn kappale -taudilla on siis todennäköisesti kaksi neuropatologisesti ja geneettisesti erilaista alatyyppiä.

Neljännessä osatyössä selvitimme, miten Alzheimerin tautiin yhdistetyt 29 perimän riskilokusta liittyvät erityyppisiin Alzheimerin taudin neuropatologisiin muutoksiin. Tutkitut neuropatologiset muutokset olivat: 1. amyloidi β-peptidin kerääntyminen hermosolujen ulkopuolelle (CERAD score), 2, amyloidi β-peptidin kertyminen verisuonien seinämiin, 3. amyloidi β-peptidin kertyminen hiussuoniin ja 4. tau-proteiinin kerääntyminen hermosolujen sisään (Braak stage). Havaitsimme, että osalla Alzheimerin taudin perimän riskikohdista oli yhteys kaikkiin eri neuropatologisiin muutoksiin (esimerkiksi *APOE*), kun taas osalla oli yhteys vain tiettyyn neuropatologiseen muutokseen. *CASS4*-, *CLU*-, ja *ZCWPW1*-geenien muutokset liittyivät spesifisti amyloidi β-peptidin kertymiseen verisuonien seinämiin. Amyloidi β-peptidin kertyminen hiussuoniin liittyi *TREM2*-geeniin. *TREM2* liittyy puolustussolujen aktivaatioon ja löydöksemme hiussuoniin kertyvän amyloidi β-peptidin suhteen saattaa selittyä riskialleelien vaikutuksella immuunijärjestelmän solujen toimintaan.

Tämä väitöskirjatyö 1. tarkensi tietämystä *C9orf72*-geenissä olevan toistojakson normaalivaihtelusta ja tautia aiheuttavasta toistojaksojen määrästä, mikä auttaa diagnostiikassa ja ALS:in tautimekanismien ymmärtämisessä, 2. osoitti, että Lewyn kappale –tautimuutokset etenevät kahdella tavalla viitaten kahteen geneettiseltä alttiudeltaankin erilaiseen alatyyppiin, jotka ovat tärkeä erottaa Lewyn kappale -taudin jatkotutkimuksissa ja mahdollisesti potilaiden hoidossa, 3. ja näytti, että Alzheimerin tautiin liitettyjä perimän muutoksia voidaan yhdistää tiettyihin neuropatologisiin muutoksiin, mikä auttaa perimämuutosten välittämien tautimekanismien selvittämistä.

ABBREVIATIONS

123I-MIBG	123I-meta-iodobenzylguanidine
ABCA7	ATP binding cassette subfamily A member 7
ABCG1	ATP-binding cassette sub-family G member 1
ABI3	ABI family member 3
ACE	Angiotensin I converting enzyme
ADAM10	ADAM metallopeptidase domain 10
ADAMTS4	ADAM metallopeptidase with thrombospondin type 1 motif 4
ADGRG	Adhesion G Protein-Coupled Receptor G1
AFR	African
ALPK2	Alpha kinase 2
ALS	Amyotrophic lateral sclerosis
AMR	Admixed American
APH1B	Aph-1 homolog B, gamma-secretase subunit
APOE	Apolipoprotein E
APP	Amyloid Beta Precursor Protein
ASO	Antisense oligonucleotide
ATP	Adenosine triphosphate
Aβ	Amyloid β
BCL7C	BAF Chromatin Remodeling Complex Subunit
BIN1	Bridging integrator 1
C9orf72	Chromosome 9 open reading frame 72
CAA	Cerebral amyloid angiopathy
CapAβ	Capillary amyloid β
CASS4	Cas scaffold protein family member 4
CD2AP	CD2 associated protein
CD33	CD33 molecule
CELF1	CUGBP Elav-like family member 1
CERAD	The Consortium to Establish a Registry for Alzheimer's Disease
CEU	Utah Residents with Northern and Western European Ancestry
CLNK	Cytokine dependent hematopoietic cell linker

CNS	Central nervous system
CNTNAP2	Contactin associated protein 2
CNV	Copy number variation
CR1	Complement C3b/C4b receptor 1 (Knops blood group)
CSF	Cerebrospinal fluid
CT	Computed tomography
DAT	Dopamine transporter
DLB	Dementia with Lewy bodies
DNA	Deoxyribonucleic acid
EAS	East Asian
ECHDC3	Enoyl-CoA hydratase domain containing 3
EPHA1	EPH receptor A1
EUR	European
EXOC3L2	Exocyst Complex Component 3 Like 2
fALS	Familial ALS
FERMT2	Fermitin family homolog 2
FIN	Finns
FTLD	Frontotemporal lobar degeneration
FUS	Fused in Sarcoma/FUS RNA binding protein
GAB2	GRB2 Associated Binding Protein 2
GABRB3	Gamma-aminobutyric acid type A receptor subunit beta3
GALNT7	Polypeptide N-Acetylgalactosaminyltransferase
GBA	Glucocerebrosidase
GBR	British in England and Scotland
gnomAD	The Genome Aggregation Database
GWAS	Genome-wide association study
HESX1	HESX homeobox 1
HLA-DRB1	Major histocompatibility complex, class II, DR beta 1
IBS	Iberian in Spain, TSI
INPPD5	Inositol Polyphosphate-5-Phosphatase D
KAT8	Lysine acetyltransferase 8
LAPTM4B	Lysosomal-associated transmembrane protein 4B

LD	Locus disequilibrium
LOSMoN	Late-Onset Spinal Motor Neuronopathy
LRP	Lewy-related pathology
MAF	Minor allele frequency
MAPT	Microtubule-associated protein tau
MEF2C	Myocyte-specific enhancer factor 2C
MLH1	MutL homolog 1
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MS4A	Membrane-spanning 4A
MSR1	Macrophage Scavenger Receptor 1
NFT	Neurofibrillary tangle
NGS	Next-generation sequencing
NIA-AA	National Institute on Aging-Alzheimer's Association
NIA-RI	The National Institute on Aging and Reagan Institute
NME1	Nucleoside Diphosphate Kinase
NME8	NME/NM23 Family Member 8
NYAP1	Neuronal Tyrosine Phosphorylated Phosphoinositide-3-Kinase Adaptor 1
PCA	Principal component analysis
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PDZD2	PDZ Domain Containing 2
PET	Positron emission tomography
PICALM	phosphatidylinositol binding clathrin assembly protein
PNS	Peripheral nervous system
PRS	Polygenic risk score
PSEN1	Presenilin 1
PSEN2	Presenilin 2
PTK2B	Protein tyrosine kinase 2 beta
QQ	Quantile-quantile plot
RBD	REM sleep behavioral disorder

RNA	Ribonucleic acid
sALS	Sporadic ALS
SAS	South Asian
SCIMP	SLP adaptor and CSK interacting membrane protein
SMN1	Survival motor neuron 1
SMN2	Survival motor neuron 2
SNCA	Synuclein alpha
SNP	Single nucleotide polymorphism
SNV	Single nucleotide variant
SOD1	Superoxide dismutase 1
SORL1	Sortilin related receptor 1
SPAG9	Sperm Associated Antigen 9
SPECT	Single-photon emission computed tomography
SPI1	Spi-1 proto-oncogene
SPTBN1	Spectrin Beta, Non-Erythrocytic 1
STR	Short tandem repeat
STX1B	Syntaxin 1B
SV	Structural variant
TARDBP	Transactive response DNA binding protein 43 kDa
TDP-43	TAR DNA binding protein 43
TREM2	Triggering receptor expressed on myeloid cells 2
TRIP4	Thyroid Hormone Receptor Interactor 4
TSI	Toscani in Italy
UK	United Kingdom
WES	Whole exome sequencing
WGS	Whole genome sequencing
WHO	World Health Organisation
ZCWPW1	Zinc finger CW-type and PWWP domain containing 1

1. INTRODUCTION

The human nervous system is complex and can be divided into the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS includes the brain and spinal cord and PNS the peripheral nerves that e.g. control muscles. Not all types of neurons are affected in one neurodegenerative disease, instead, typically specific neuron populations are affected. Thus, the clinical spectrum of neurodegenerative diseases is vast and reflects the parts of the nervous system that are affected by pathogenic processes. For example, in amyotrophic lateral sclerosis (ALS) the motor nerves in the CNS and PNS die leading to the gradual loss of voluntary movements. However, cognition remains unaffected in ALS patients if the brain regions involved in cognitive processes are left intact. On the other hand, in Alzheimer's disease (AD) where the neurons of the hippocampus and entorhinal cortex are among the first to be affected, cognition and memory are impaired but movements can remain normal. In dementia with Lewy bodies (DLB), there is both loss of cognition and impairment of movements.

Neurodegeneration can be caused by many factors, both intrinsic and extrinsic. It is a slow process and can take from months to decades. For example, alcohol abuse leads to changes in both the CNS and PNS in the forms of cerebellar degeneration and peripheral neuropathy. In AD, the preclinical stage of the pathological evolution often starts in middle age (40-50s) whereas patients are usually diagnosed after their late sixties (Kok et al., 2009; Rajan, Wilson, Weuve, Barnes, & Evans, 2015).

The high number of potential causes, the extensive time the disease takes to develop, and the complexity of the nervous system have made the study of neurodegenerative diseases challenging. Neuropathological studies that characterize the changes in the nervous system have been crucial in building modern understanding of neurodegenerative diseases. For example, Alois Alzheimer described amyloid β (Aβ) depositions in the brain in 1906, and in 1962 the topography of neurofibrillary structures were reported (Hirano & Zimmerman, 1962). In 1986, phosphorylated tau was identified as the key component in neurofibrillary tangles (Kosik, Joachim, & Selkoe, 1986). Later, genetic studies tied together the neuropathological observations to show that the observed neuropathological changes can be attributed to genetic mutations, helping to explain the pathogenic mechanisms on a molecular level. Important milestones were the purification and characterization of Aβ in 1984 (Glenner & Wong, 1984) and the cloning of the *APP* gene in 1987 (Kang et al., 1987). The first *APP* mutations found to cause early-onset AD were reported in 1991, with *PSEN1* and *PSEN2* mutations in 1995 (Chartier-Harlin et al., 1991; Levy-Lahad et al., 1995; Sherrington et al., 1995).

During the last decade, the role of genetics in research has become increasingly important thanks to new technologies. Instead of studying families with specific high impact mutations, researchers have also been able to study the genetics of thousands of cases and controls simultaneously. These genome-wide association studies have allowed for the identification of important loci in complex diseases such as schizophrenia (Schizophrenia Working Group of

the Psychiatric Genomics Consortium, 2014) where there is no one major mutation but many variants across multiple loci together with environmental factors affecting the phenotype. When genome-wide association studies are conducted on next-generation sequencing data, it can allow rapid detection of causal variants in a population.

Genetics as a field has advanced tremendously over the last few decades: the human genome project took over a decade and over 3000 million US dollars whereas now a whole genome can be sequenced in a day for under 2000 dollars. Despite this progression and significant effort, disease-modifying treatments for neurodegenerative diseases are scarce. To date, the vast majority of interventions are symptomatic: they can lessen the symptoms but cannot alter the course of the disease. However, with simultaneous advancements in pathological methods, imaging technologies and the continuing advancements in genetics, neurodegenerative diseases can be studied in a much more comprehensive manner than before and disease-modifying treatments are on the horizon.

The aim of this work was to further study the genetics of three neurodegenerative diseases: ALS, AD and the closely related DLB. Improved knowledge of disease genetics can elucidate disease mechanisms, which can translate to a better understanding of how diseases progress and eventually to disease-modifying treatments.

2. REVIEW OF LITERATURE

2.1. The human genome and genetic variation

2.1.1. The human genome

The first draft of the human genome was published in 2001 (Gamba, 2001) and the project was finished a couple of years later (International Human Genome Sequencing Consortium, 2004). This genome sequence (build, i.e. version 35) consisted of 2.85 billion nucleotides and an estimated 20 000 – 25 000 genes, but had several hundred gaps. Since then, the reference genome has been updated and the latest major version (build 38) was released in December 2013. It consists of 3 billion base pairs on 22 autosomes, two sex chromosomes and mitochondrial DNA. Despite major improvements, there are still gaps and sequences on unlocalized chromosomal areas or scaffolds.

Only 1% of the human genome encodes proteins and the rest has been historically regarded as "junk DNA" but this belief is nowadays largely questioned. The non-coding regions and their roles in both health and disease are still largely unknown, although some important functions are known. For example, it has been reviewed that transcriptional enhancers and long non-coding RNA play important roles both in normal physiology, development and disease (Lee, H., Zhang, & Krause, 2019; Nord & West, 2019).

2.1.2. Genetic variation

The simplest and most common type of genetic variation is single nucleotide variation (SNV) or single-nucleotide polymorphism (SNP), where one base is different compared to the reference genome (Figure 1). In a typical genome, there are roughly 3-4 million SNVs (Shen et al., 2013), thus only an extremely rare number of SNVs are pathogenic. The majority of SNVs are very rare at a population level and the number of identified rare variants has increased tremendously as more genomes are sequenced. For example, in the 1000 Genomes dataset with 2500 genomes, there were 85 million SNVs from which 64 million SNVs had a minor allele frequency (MAF) <0.5% whereas there were 8 million SNVs with a MAF > 5% (1000 Genomes Project Consortium et al., 2015). In the gnomAD version 3 dataset with 20314 genomes, there were more than 200 million SNVs but the number of common variants with a MAF > 5% was roughly the same 8 million (Karczewski et al., 2020).

Indels are small (less than 50bp) insertions or deletions and like SNVs they are abundant, with roughly 0.4-0.5 million per genome (1000 Genomes Project Consortium et al., 2015). The large number of SNVs and indels pose a challenge in genetic studies trying to identify pathogenic variants since there are over 100 protein truncating variants caused by SNVs and indels per genome (Lek et al., 2016).

Larger insertions and deletions, as well as different rearrangements and copy-number variations are called structural variants (SVs). SVs are more often pathogenic than smaller variations, but they are also much rarer. In the 14891 individuals with deep whole-genome sequencing data of the Genome Aggregation Database (gnomAD), there were around 8000 SVs per genome and as a consequence, on average eight genes were altered by rare SVs (Collins et al., 2020).

Another type of variation is tandem repeat variation, e.g. short tandem repeats (STRs) where there is a repeat unit of 1-6 base pairs. STRs are almost as common as indels in the genome and there are more than 30 hereditary diseases caused by STRs, for instance Huntington's disease, fragile X syndrome and myotonic dystrophy. The repeats lead to changes in gene expression and accumulation of RNA and repeat peptides. Often the number of repeats increases in offspring leading to genetic anticipation, i.e. the disease begins earlier and is more severe in each subsequent generation (McComas, Sica, & Toyonaga, 1978; Mirkin, 2007).

The variations above change the base pair sequence of DNA but there is also variation where the DNA sequence remains intact. Epigenetic changes affect the chromatin state and thus transcription, without changing the DNA sequence. Methylation of DNA was one of the first epigenetic modifications observed (Gold, Hurwitz, & Anders, 1963), other common epigenetic changes include histone modifications (Cavalli & Heard, 2019). Epigenetic modifications are an important reminder that although DNA is often studied as a two-dimensional sequence of bases, it is a complex three-dimensional structure.

2.1.3. Genotyping and sequencing technologies

Modern sequencing technologies allow high-throughput studies with decreasing costs. Commonly used methods include genome-wide genotyping assays, whole-exome (WES) and whole-genome sequencing (WGS). Genotyping arrays include a variable number of markers preselected to capture the genetic variation with typically 0.5 – 3 million markers. Albeit genotyping chips rarely include pathogenic variants, they are a cost-effective way of conducting genome-wide association studies and some arrays also enable large CNV detection. Furthermore, genotyping arrays can be used to impute missing genotypes. Imputation is a method where missing genotypes are inferred based on haplotypes from a reference sample. Imputation is very accurate with common (MAF > 5%) variants but good results can be achieved for rare variants as well, especially when using sufficiently large ancestry-matched reference panel (Mitt et al., 2017).

WES targets the exons, i.e. the protein coding part of the genome. Since many disease-causing variants are exonic, it is a more cost-effective way compared to WGS to find likely pathogenic mutations in Mendelian diseases. As costs come down, WGS has become widely used. It also covers non-exonic regions and gives a much more detailed picture on individual genetic variation. However, the plethora of variants poses a problem for variant prioritization. There

are established guidelines for determining the likelihood of the pathogenicity of variants in exonic regions (Richards et al., 2015), but the classification of non-coding variants is still difficult. Typically, short-read WGS is used and allows for excellent detection of SNVs, indels and performs well with some structural variants. However, to more reliably capture complex structural variants such as complex rearrangements or tandem repeats, it is advisable to use long-read WGS (Lappalainen, Scott, Brandt, & Hall, 2019).

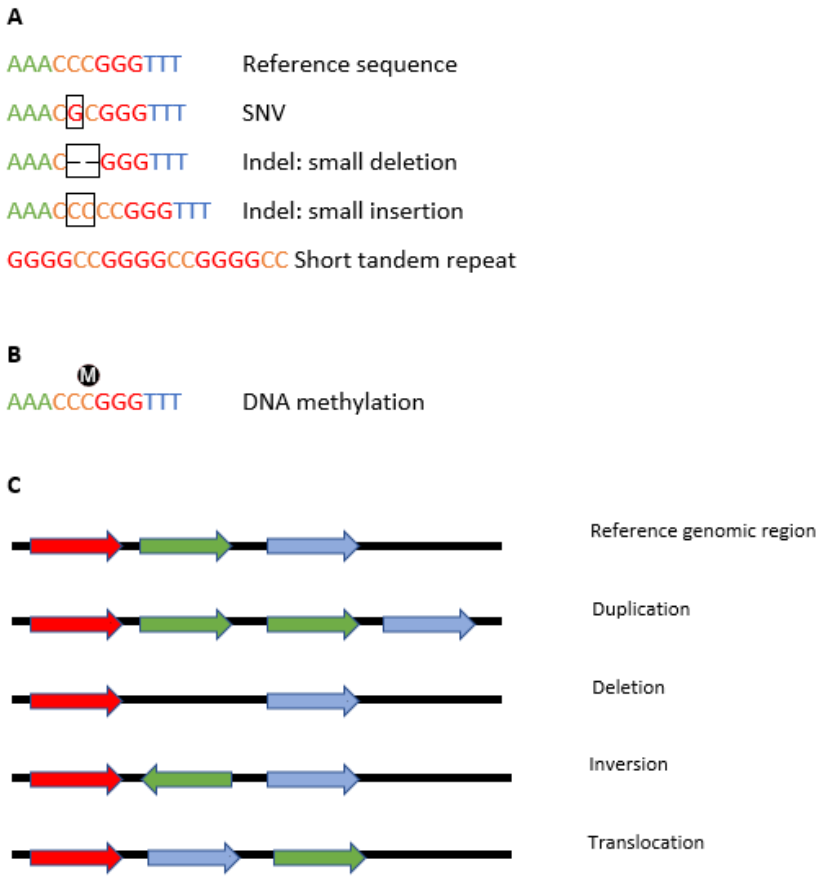

Figure 1: A) Examples of small genetic variations. First is an imaginary reference sequence, followed by an SNV where there is a point mutation of a single base. Following these, a small insertion and a small deletion are shown, followed by a short tandem repeat (GGGGCC). B) DNA methylation at GpC does not change the base pair sequence. C) Examples of larger structural variants at an imaginary genetic region.

Currently, short-read sequencing and sequencing-by-synthesis is the most widely used method of next-generation sequencing (NGS). A typical sequencing workflow includes four steps. First, library preparation includes DNA fragmentation into smaller pieces to which adapters are ligated. The second step is known as cluster generation where the library is put on the sequencing machine's flow cell and adapters on the DNA bind to the surface of the flow cell. Clusters, i.e. groups of "clones" of attached sequences are generated using bridge amplification. The third step is the sequencing where the bases are identified based on the signal they send. Cluster formation enables the signal to be strong enough for accurate capture. The final step is the data analysis, where quality control of the sequencing reads is performed and the usually billions of sequencing reads are aligned to the reference genome for identification of genetic variants.

2.1.4. Genome-wide association studies

Genome-wide genotyping and sequencing technologies allow genome-wide association studies (GWAS) (Visscher et al., 2017). In a GWAS, typically hundreds to thousands of unrelated individuals are studied in order to find loci that associate with different phenotypes. The results are often presented as a "Manhattan plot" (Figure 2). However, even if a statistically significant association is found, the effect size or odds ratios of an individual locus on the phenotype tend to be small (typically < 1.3) in complex diseases. Still, when the effect of many individual loci with small effects are considered together, the loci can explain together a significant amount of phenotypic variance. For example, adult height is a complex polygenic trait in which circa 700 variants explain reportedly 20% of its heritability (Wood et al., 2014). One application of summarizing the effects of individual loci is the polygenic risk score (PRS) that can be calculated for various phenotypes. Moreover, the identification of new loci even with small effect sizes can give valuable information on diseases via pathway analysis. Pathway analysis is based on prior knowledge of gene function. For example, loci associated in a recent large AD genome-wide association analysis were enriched in pathways involved in e.g. degradation of amyloid precursor proteins and lipid metabolism (Jansen et al., 2019).

However, the association between an identified locus and the biology of the disease is often complex and although GWASes have successfully identified thousands of loci, how the observed associations translate into disease is often unknown. After sufficient replication to find true associations, functional "post-GWAS" studies are needed to elucidate the role of observed variants (Gallagher & Chen-Plotkin, 2018). One major hurdle in understanding the effects of GWAS results is that the top association is rarely the causal variant since variants on genotyping arrays are mostly selected based on haplotype structure. Rather, due to linkage disequilibrium (LD), it flags the haplotype that associates with the disease. Moreover, if the top hit is in an intergenic area, the gene of interest can be unclear. One example of a common post-GWAS functional method is to study the overlap of a putative causal variant with the expression levels of different genes. This is especially useful if the suspected causal variant is in the non-coding region, as many are (Maurano et al., 2012). With the increasing

development of gene editing technology, the effects of haplotypes or individual SNPs can also be studied in different cell types (Gallagher & Chen-Plotkin, 2018).

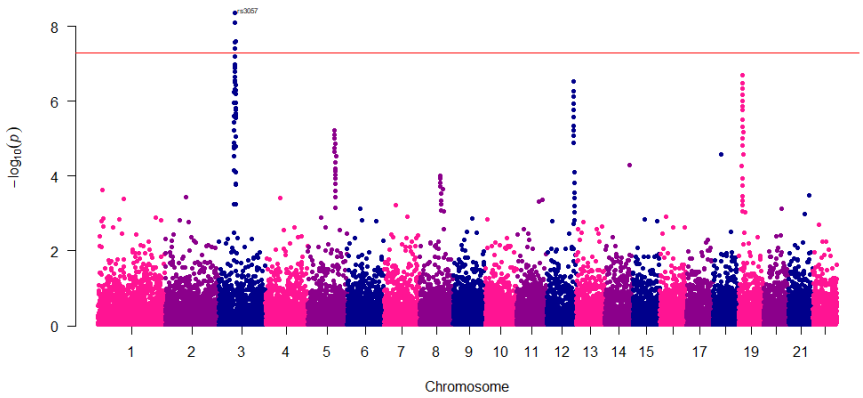

Figure 2: Manhattan plot. On the y-axis is the –log10 of the P-value and on the x-axis are the genomic positions of every variant per chromosome. Each dot represents one variant. In GWAS, due to multiple testing correction, only p-values less than $5x10^{-8}$ are regarded as statistically significant. This threshold is represented as a horizontal line. The name of the plot is a reference to the skyline of Manhattan with the association peaks as skyscrapers. This picture is based on the "gwasResults" data supplied with the "qqman" package of R (Turner, S.D. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots.)

2.1.5. Finnish population genetics

Finnish population genetics have some unique features owing to our history and how people settled in Finland. There are many theories about how migration, agriculture, wars etc. affected the early Finnish population. Be that as it may, the genetic composition today is most likely affected by a founder effect in addition to several bottlenecks caused by epidemics and famine. Geographical isolation has made Finns a genetically homogenous population but e.g. there is a notable east-to-west difference that has been extensively reviewed (Norio, 2003b; Palo, Ulmanen, Lukka, Ellonen, & Sajantila, 2009), as well as regional subpopulations (Kerminen et al., 2017). The east-to-west difference has been suggested to at least partially reflect the two main waves of settlement: first the western parts along the coast, then to the eastern and northern regions (Norio, 2003b).

From a genetic perspective, Finns are outliers of European populations, as are people from Iceland and some other populations situated geographically on the borders of Europe.

Genotyping some 50 000 - 200 000 SNPs already enables visualization of European genetic structures using principal component analysis (PCA). However, in a global context, Finns clearly cluster with other European populations (Figure 3). Modern technologies paired with population registry data allow much more precise analyses on genetic structure than standard nation-level comparisons. The Finnish genetic structure was fine mapped in 2017 (Kerminen et al., 2017). It showed that the fine structure is strongly geographically clustered across Finland and that the main split is indeed the historical east-west axis. Knowing the fine structure has its own academic value but it also has applications e.g. in rare variant association studies where population substructure can become a confounding factor (Mathieson & McVean, 2012).

The Finnish disease heritage is one example of the unique Finnish population genetics. The Finnish disease heritage consists of over 30 diseases that are seen in Finland but are rare or absent in other populations such as cartilage-hair dysplasia and polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy. Most of these diseases are inherited recessively and for most the genetic cause is also known. On the other hand, some diseases such as cystic fibrosis are considerably rarer in Finland than elsewhere (Norio, 2003a).

In addition to Finnish disease heritage, some other pathogenic variations of more common diseases are overrepresented in Finland. Examples include the *C9orf72* hexanucleotide repeat expansion that causes ALS and frontotemporal lobar degeneration (FTLD) (Majounie, Renton et al., 2012) and the deletion of exon 16 in *MLH1* in hereditary nonpolyposis colon cancer (Lynch & de la Chapelle, 1999).

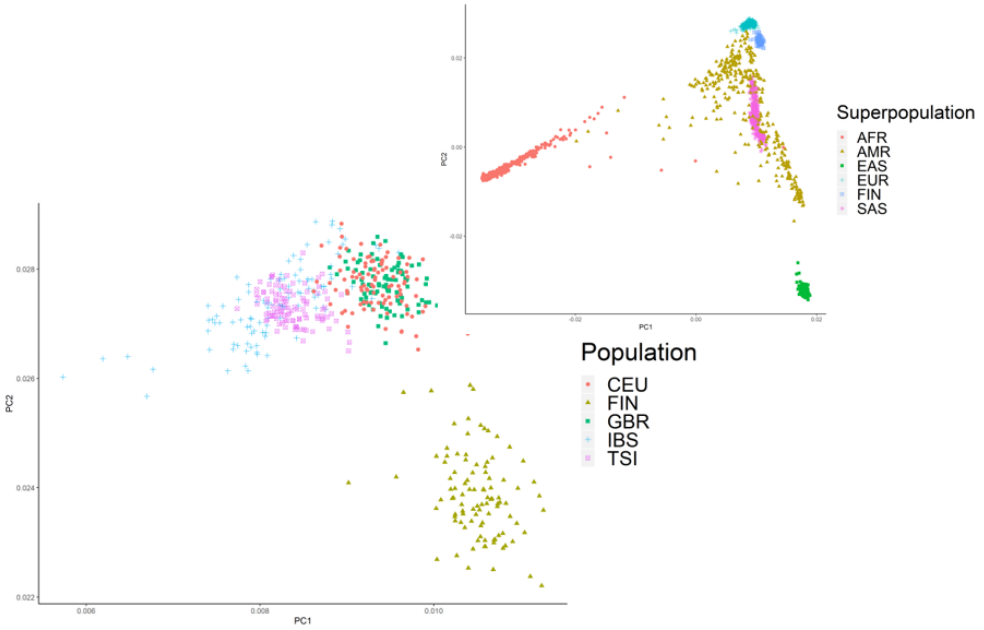

Figure 3: PCA of European populations and smaller picture of global PCA. On the x-axis is the 1st principal component and on the y-axis the 2nd. Each dot represents one individual. On the lower big plot are individuals of European ancestry and Finns form a separate cluster. However, in a global context, Finns cluster with other Europeans as seen in the smaller plot. Populations: CEU = Utah Residents with Northern and Western European Ancestry, FIN = Finns, GBR = British in England and Scotland, IBS = Iberian in Spain, TSI = Toscani in Italy. Superpopulations: AFR = African, AMR = Admixed American, EAS = East Asian, EUR = European, FIN = Finns, SAS = South Asian. Data from phase III of the 1000 genomes project (1000 Genomes Project Consortium et al., 2015).

2.2. Aging and neurodegeneration

Aging, the gradual decline of an organism's function over time, is an inevitable part of life. From a broader sense, aging is caused by cellular damage (Kirkwood, 2005). Aging is a very complicated process but the accumulation of cellular damage has been summarized as being due to nine partially overlapping hallmarks: genomic instability, telomere shortening, epigenetic alterations, loss of protein homeostasis (proteostasis), deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence (cell cycle arrest, inability to produce daughter cells), stem cell exhaustion and altered intercellular communication (Lopez-Otin, Blasco, Partridge, Serrano, & Kroemer, 2013) (Figure 4). Since aging is the strongest risk factor for many neurodegenerative diseases (Duggan, Torkzaban, Ahooyi, Khalili, & Gordon, 2019), it is natural that there is considerable overlap between the mechanisms of neurodegeneration and aging. Furthermore, the brain weighs roughly 1300 grams or 2% of

the body's mass but consumes about 20% of our daily energy (Rolfe & Brown, 1997). Due to this high metabolic rate, neurons are susceptible to pathological changes in cell metabolism that lead to cellular damage and neurodegenerative diseases. Moreover, neurons are post-mitotic by nature, which resembles permanent cell cycle arrest, i.e. cellular senescence, one of the hallmarks of aging.

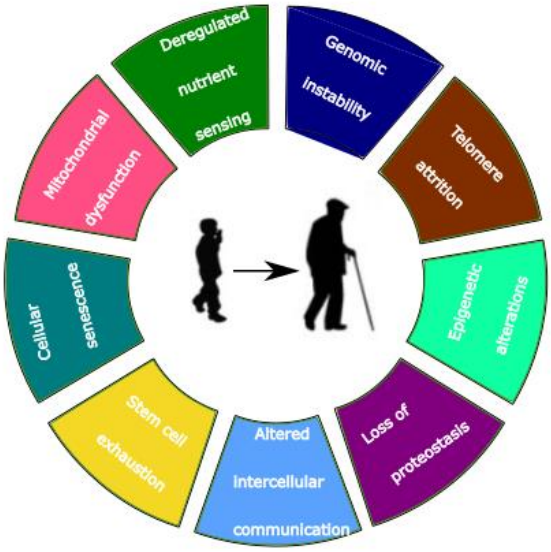

Figure 4. The hallmarks of aging. The different hallmarks are not separate entities but overlap. The hallmarks are present in normal aging, and their amelioration or aggravation slow down or accelerate aging, respectively. Both endogenous and exogenous processes affect aging. For example, mutations in DNA occur in normal DNA replication but UV-radiation can also cause them. Many of these hallmarks are the same as the molecular mechanisms behind neurodegeneration. Image redrawn and modified from (Lopez-Otin et al., 2013).

Neurodegenerative diseases can be classified in various ways, e.g. by the predominant symptom or location of the predominant lesion (Przedborski, Vila, & Jackson-Lewis, 2003). Although many neurodegenerative diseases show considerable overlap and symptoms vary, dementia is one major endpoint of neurodegeneration. WHO has estimated that in 2030 there will be more than 80 million people with dementia and this number increases to 150 million in 2050. In 2015, WHO estimated that dementia leads to a cost of over 800 billion dollars and this cost will increase to over 2000 billion dollars in 2030 (https://www.who.int/news-room/fact-sheets/detail/dementia). The humane cost of dementia is in the loss of good quality life years of the patient and the toll of care and worry by relatives.

Despite dementia being a major endpoint (and perhaps the best-known one for larger audiences), not all neurodegenerative diseases lead to dementia, rather, clinical features reflect the damaged part of the nervous system. The symptoms vary based on the affected regions and do not always include dementia or cognitive disturbances but rather muscle weakness, spasticity, muscular atrophy or rigidity as in ALS, spinal muscular atrophy and different ataxias (Table 1).

Table 1. The most severely affected regions of the nervous system in different neurodegenerative diseases.

Disease	**Most severely affected region**
Alzheimer's disease	Cerebral cortex (medial temporal lobe)
Dementia with Lewy bodies	Cerebral cortex, amygdala, substantia nigra
Parkinson's disease	Substantia nigra
Amyotrophic lateral sclerosis	Motor neurons and interneurons (CNS and PNS)
Frontotemporal dementia	Frontal and temporal lobes
Polyneuropathies	Peripheral motor and sensory neurons
Spastic paraplegias	Motor neurons (CNS)
Spinocerebellar ataxias	Cerebellum (spinal cord)

Neurodegeneration is often accompanied by the accumulation of misfolded proteins that demonstrates the dysfunction of proteostasis. Neurodegenerative changes accumulate with age and are present even in cognitively unimpaired individuals more as a rule than as an exception. A summary of pathological studies concluded that in the brains of individuals older than 80 years, hyperphosphorylated tau was present in over 90% and Aβ in over 60% and the prevalence of mixed pathology also increased with age (Elobeid, Libard, Leino, Popova, & Alafuzoff, 2016). There is often, however, a distinction between changes seen in “normal” aging versus pathological neurodegeneration. In disease, neurodegeneration is considered to be present in major neuronal circuits and there is cell specific vulnerability (Morrison & Hof, 1997). For example, in mild AD there is already a loss of up to 50% of neurons in layer II of the entorhinal cortex compared to no loss in neurologically healthy individuals (Morrison & Hof, 1997).

Whether the different proteins that accumulate in the brain are a by-product of neurodegenerative processes or their cause remains unclear. Nevertheless, it has been reviewed that the accumulation of such proteins points to problems in protein degradation as a key mechanism not only in aging but also in neurodegeneration (Haass & Selkoe, 2007).

Besides changes in protein homeostasis, the mechanisms of neurodegeneration are many and are thought to include for example DNA damage, lysosomal dysfunction, epigenetic changes and immune dysregulation (Katsnelson, De Strooper, & Zoghbi, 2016). To some extent, these are a part of normal aging but in disease these mechanisms are altered or advance at a much faster pace than normal.

As previously stated, there is considerable overlap between the mechanisms of aging and neurodegeneration and genetics has had an important role in studying the two phenomena. Apolipoprotein E (*APOE*) was one of the main associations in recent longevity meta-analysis (Deelen et al., 2019) and it has also been associated with several neurodegenerative diseases such as AD (Strittmatter et al., 1993) and DLB (Guerreiro et al., 2018) but also in cardiovascular and renal diseases (Feussner et al., 1992; Ghiselli, Schaefer, Gascon, & Breser, 1981). It has three main genotypic variants: ε2, ε3 and ε4. ε3 encodes the major isoform followed by ε4. ε4 is the disease associated variant, whereas ε2 is protective and ε3 is regarded as neutral. There is also evidence that certain regulatory variants at the *APOE* locus modulate the risk of AD and cognitive decline, independent of the protein polymorphism (Myllykangas et al., 2002; Rantalainen et al., 2016). *APOE* mediates lipid transfer but also binds to different inflammatory molecules and contributes to immune responses (Ali, Middleton, Pure, & Rader, 2005).

2.3. Dementia with Lewy bodies

2.3.1 Definition and epidemiology

DLB has not been recognized as a separate and distinct disease for long; only in 1984 was it proposed as its own disease entity (Kosaka, Yoshimura, Ikeda, & Budka, 1984) and the first international consensus criteria for its diagnosis were published in 1996 (McKeith, I. G. et al., 1996).

DLB is a synucleinopathy, a neurodegenerative disorder characterized by the accumulation of α-synuclein. A subcategory of synucleinopathies are Lewy body diseases where α-synuclein aggregates into Lewy bodies and Lewy neurites, collectively called Lewy-related pathology (LRP). The most common Lewy body diseases have been reviewed to be DLB, PD and Parkinson disease dementia (PDD) (Jellinger, 2003).

As can be deduced from its late discovery, DLB often clinically resembles other neurodegenerative diseases such as AD and PD and it can sometimes be differentiated by PDD only by “the one year rule”. This rule dictates that in DLB dementia precedes or is concomitant with parkinsonism whereas in PDD dementia begins at least one year later than motor symptoms (Yamada et al., 2019). DLB has been “overshadowed” by AD and PD and this has led to poor general knowledge of the disease and also under-diagnosis of DLB (Vann Jones & O'Brien, 2014) that has held back research.

With increasing awareness and knowledge of the disease, DLB is now considered the second most common primary dementia after AD but estimates of its prevalence vary greatly. In population studies of those over 65 years, mean prevalence was 0.36% whereas <7.5% in clinic-based studies (Vann Jones & O'Brien, 2014). In Finland, the largest prevalence study of

DLB was published in 2003 (Rahkonen et al., 2003). They studied 601 individuals from the Kuopio region, aged at least 75 years and found the prevalence of DLB to be 5% or 22% among all individuals with dementia. For comparison, in the same study, the prevalence of AD was 10.6% representing 47% of all dementias. LRP is much more common than actual DLB and it was present in more than a third of Finnish individuals aged over 85 years (Oinas et al., 2009).

Risk factors for DLB seem to partly overlap with those of AD and PD. The strongest risk factor is advanced age, and DLB is more common in men than in women. Family history of dementia, a history of depression and anxiety increased the risk of DLB, whereas caffeine use and a history of cancer reduced it (Boot et al., 2013). Whilst lower education correlates with AD, higher education correlates with DLB and PD (Guerreiro et al., 2019).

2.3.2. Neuropathology and molecular mechanisms

A key molecular mechanism behind DLB like other synucleinopathies is thought to be the abnormal aggregation of α-synuclein. α-synuclein is coded by the *SNCA* gene on chromosome 4. The initial nucleation process is the rate-limiting step of fibril formation but preformed oligomers and fibrils can speed up formation of new aggregates (Iljina et al., 2016). The mature fibrils might not be behind toxicity but the pathological changes and eventual neuronal loss could be due to heterogeneous oligomers, of which some have been shown to be highly neurotoxic (Grassi et al., 2018). It has also been shown that α-synuclein can spread from cell-to-cell much like prions (Desplats et al., 2009). Despite considerable effort, the normal physiological function of α-synuclein is not clearly understood. α-synuclein knock-out mice are viable and do not show evident neurodegeneration early on but there are changes at the synapses that increase with age (Greten-Harrison et al., 2010). Therefore, in disease development, the overexpression and misfolding of α-synuclein has been upheld as important (Thakur, Chiu, Roeper, & Goldberg, 2019).

In tissues, α-synuclein can be visualized using immunohistochemistry, which allows the detection of LRP. Loss of dopaminergic neurons in the substantia nigra, Lewy neurites and intracytoplasmic Lewy bodies in surviving neurons are key neuropathological changes in DLB and PD (McKeith, I. G. et al., 1996). In DLB, there are often also widespread neocortical Lewy bodies. With the new 5G4 α-synuclein antibody, Lewy neurites are also observed in areas that have not yet developed Lewy bodies (Kovacs et al., 2012). Based on a semiquantitative approach, LRP can be staged or categorized in several ways. Two widely used classification schemes are Braak staging (Braak et al., 2003) and the DLB Consortium classification (McKeith, I. G. et al., 2017). In Braak staging, LRP is thought to progress caudo-rostrally i.e. from brainstem towards neocortex. However, this staging fails to explain increasing observations that LRP can be seen only in limbic regions, especially in adjunction with AD pathology (Dickson, Uchikado, Fujishiro, & Tsuboi, 2010; Hamilton, 2000). These

observations have raised speculations of a distinct AD associated synucleinopathy (Uchikado, Lin, DeLucia, & Dickson, 2006). The new DLB consortium classification acknowledged these observations by adding an amygdala predominant group but categorized it as a low likelihood sign of DLB. To date, it is not known, how common this AD associated synucleinopathy is in the general population since previous studies have used disease cohorts or study participants were recruited from specialized centers, leading to referral bias. It is also unknown if there are genetic differences that separate this LRP type from other LRP types and why some develop it and others do not (Figure 5).

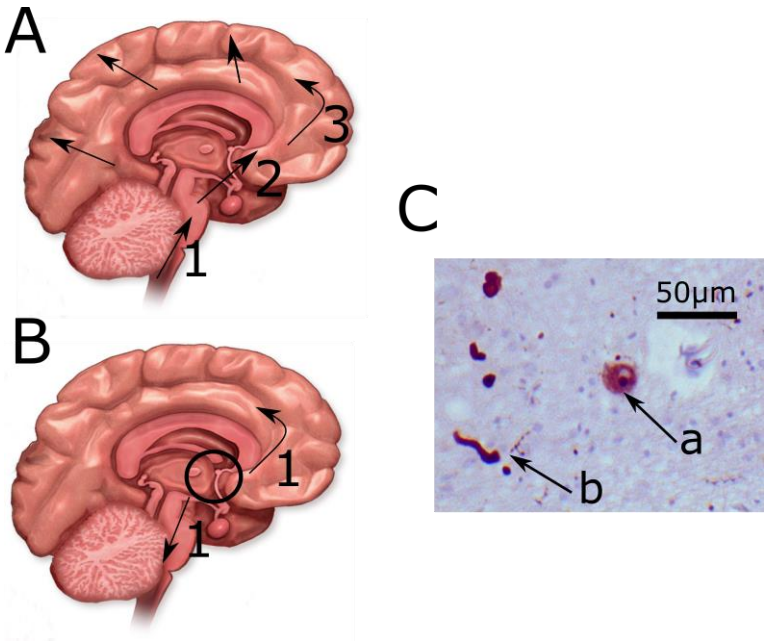

Figure 5. A. Caudo-rostral progression of LRP and B. hypothesized amygdala-based spreading in the "Alzheimer associated synucleinopathy". C. Microscopic image of LRP where arrow a shows a Lewy body and arrow b a Lewy neurite. Original picture "Photomicrographs of regions of substantia nigra in this Parkinson's patient show Lewy bodies and Lewy neurites in various magnifications" by Suraj Rajan modified and reprinted under the creative commons license CC BY-SA 3.0.

It is noteworthy that the mixed pathologies of AD and DLB most likely do not develop irrespective of one another (Figure 6). It has been shown that overexpression of α-synuclein can promote beta-secretase activity, leading to enhanced cleavage of Aβ from its parent protein increasing the secretion of Aβ (Roberts, Schneider, & Brown, 2017). Moreover, it has been observed that transgenic mice that express both human Aβ peptide and α-synuclein

develop prominent neurodegeneration and enhanced α-synuclein accumulation (Masliah et al., 2001). Furthermore, the injection of α-synuclein fibrils in mice with prominent Aβ plaque pathology clearly accelerated α-synuclein pathology. This led to spreading of α-synuclein pathology and neuron loss (Bassil et al., 2019). In neurodegenerative diseases with mixed pathologies, the different pathological features seem to interact and can form a vicious cycle where they enhance one another. Due to the high prevalence of *APOE* ε4 in AD and common co-occurrence of AD and DLB, the effects of *APOE* on α-synuclein pathology is under intense investigation. Of special interest is whether *APOE* is an independent driver of α-synuclein pathology. There are both mouse and human studies that support this hypothesis. Two recent studies that used human α-synuclein transgenic mice expressing different human *APOE* isoforms, presented evidence suggesting *APOE* ε4 regulates synucleinopathies directly and independently of amyloid deposition (Davis et al., 2020; Zhao et al., 2020). There are also human studies that reported *APOE* association with LBD independent of AD pathology (Dickson et al., 2018; Tsuang, D. et al., 2013; Zhao et al., 2020). However, the sample sizes of these studies have been small and used variable inclusion and exclusion criteria. Moreover, there are some studies that did not observe *APOE* associating with DLB independent of AD co-pathology (Prokopenko et al., 2019; Schaffert et al., 2020) so as is yet there is no definitive answer on the role of *APOE* in α-synuclein pathology.

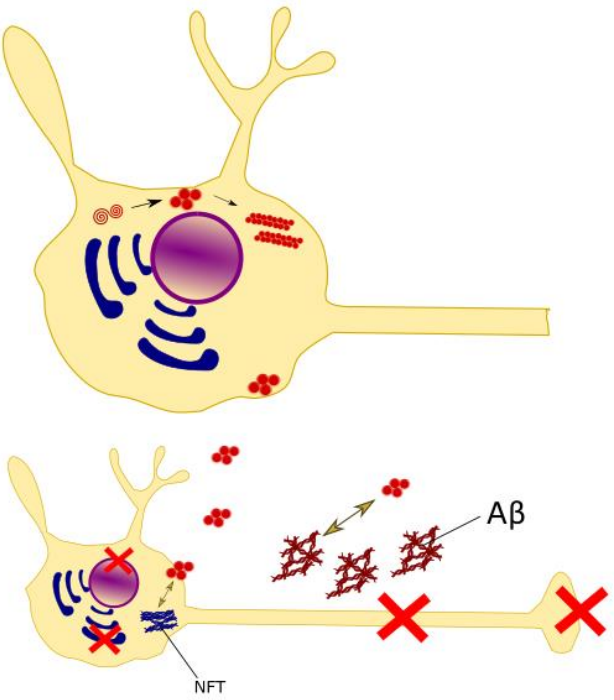

Figure 6: Possible molecular mechanisms of DLB. α-synuclein forms monomers (red spiral) that aggregate into oligomers (single red sphere). Oligomers assemble into fibrils (aggregates of red spheres). The process leads to cellular dysfunction and synaptic dysfunction (red crosses), cell death and neurodegeneration but the exact mechanisms are unknown. α-synuclein can also spread from cell-to-cell like prions via an unknown mechanism and can also interact with amyloid β (Aβ) and neurofibrillary tangles (NFT) to form a vicious cycle leading to increased pathology.

2.3.3. DLB Genetics

Despite its relatively high prevalence, the genetics of DLB is poorly characterized compared to AD and PD. Having siblings with DLB raises the odds of getting it to over two-fold (Nervi et al., 2011), which points to a genetic etiology. However, reports of familial DLB are rare and the clinical manifestations vary within and between DLB families, and usually the pathological mutation cannot be ascertained (Anderson et al., 2006; Clarimon et al., 2009; Galvin et al., 2002; Meeus et al., 2010; Tsuang, D. W. et al., 2002). Mutations identified in familial DLB overlap with AD and PD mutations and include e.g. *SNCA* duplication (Obi et al., 2008), *APP* duplication (Guyant-Marechal et al., 2008), and *SNCA* E46K missense mutation (Zarranz et

al., 2004) and *PSEN2* missense mutation (Piscopo et al., 2008). However, it is important to note that most genetic studies on familial DLB were done prior to routine WGS and advances in bioinformatics that have greatly improved the discovery of causal variants.

Indeed, in recent years and with newer sequencing technologies significant progress has been made in unravelling the genetics of DLB. One of the first GWAS of neocortical LRP was conducted in Finland in the Vantaa85+ study and identified two putative loci: *SPTBN1* and *HLA-DPB1* (Peuralinna et al., 2015). The locus near *SPTBN1* was replicated in a British sample. *SPTBN1* is of special interest since it encodes a form of β-spectrin that is expressed in the CNS and has also been found in Lewy bodies and interacts with α-synuclein (Lee, H. J., Lee, & Im, 2012; Leverenz et al., 2007).

To date, the largest GWAS of DLB consisted of 1743 cases (1324 pathologically confirmed) and 4454 controls (Guerreiro et al., 2018). In this two-stage study, significant associations were found in *APOE, SNCA, BCL7C/STX1B, GABRB3* and *GBA*. Associations with *APOE, SNCA*, and *GBA* were also replicated in the second stage of the study. This GWAS was a landmark study in DLB genetics, but at the same time it reflects how far behind DLB genetics is compared to AD and PD research whose largest GWASes have had 26035 to 94437 cases (Chang et al., 2017; Kunkle et al., 2019).

Besides GWAS on common variants, newer studies have also studied the effects of rare variants on DLB. In one, *GBA* was also confirmed in a gene-based burden analysis studying rare variants (Guerreiro et al., 2018). Rare copy number variants have also been associated with DLB. Whole-genome genotyping of 1454 DLB cases and 1525 controls showed deletions in *SPAG9, LAPTM4B*, and *NME1* loci associated with DLB. Other identified loci with significant associations were *MSR1*, *PDZD2* and *ADGRG*. Copy number variations in *SNCA* were also identified (Kun-Rodrigues et al., 2019) in some individuals with DLB.

Despite only a few loci associated with DLB, the phenotypic variance or heritability of DLB was recently estimated to be nearly 60% (Guerreiro et al., 2019) which is roughly the same as that estimated for AD (58% - 79%) (Gatz et al., 2006) and PD (up to 70%) (Nalls et al., 2019). Rare variants explained most of the heritability. Another interesting finding of the same study was that AD and PD polygenic risk scores were significantly associated with DLB but explained only a little of the phenotypic variance (1.33% and 0.37% respectively) despite the three best-replicated risk loci of DLB also being major risk loci for AD (*APOE*) and PD (*GBA, SNCA*). These observations suggest that many loci associated with DLB are yet to be found and require larger studies. Moreover, despite overlap in clinical features and the identification of AD and PD loci in GWAS, it seems that DLB is not simply an amalgamation of AD and PD risk factors.

2.3.4. Diagnosis and management

Diagnosis of DLB can be challenging because of clinical overlap with other neurodegenerative diseases. The DLB consortium published its fourth consensus report on diagnosis and management of DLB in 2017 (McKeith, I. G. et al., 2017).

The diagnosis of DLB requires many approaches. It has been demonstrated that REM sleep behavior disorder (RBD) often precedes other symptoms and that three fourths of autopsy-confirmed DLB patients had RBD (Ferman et al., 2011). However, in a clinical setting, there is rarely any polysomnogram data and signs of RBD must be gathered from patient history and descriptions from their partner. Other core clinical features are dementia with fluctuations in cognition, visual hallucinations and parkinsonian features. Visual hallucinations are complex and quite vivid and relate for example to people, animals and landscapes (Harding, Broe, & Halliday, 2002).

Imaging studies help diagnosing DLB, but the usefulness of routine MRI/CT is limited. In MRI/CT, patients with DLB usually have less atrophy in the medial temporal lobe than patients with AD but the sensitivity and specificity for distinguishing AD and DLB are both less than 70% (Harper et al., 2016). AD and DLB can be better distinguished with SPECT or PET where DLB has reduced DAT uptake (McKeith, I. et al., 2007). However, in patients with dementia and previous RBD, probable DLB diagnosis is possible if the polysomnography shows REM sleep without atonia (Boeve et al., 2013; McKeith, I. G. et al., 2017). Another promising biomarker with similar sensitivity is the low uptake of 123I-MIBG in myocardial scintigraphy. 123I-MIBG is similar to norepinephrine and correlates with sympathetic nerve denervation which is seen in PD and DLB (Komatsu et al., 2018).

Despite recent advances in DLB genetics, there are no reliable genetic biomarkers. As yet, fluid biomarkers unique for DLB do not exist either. Cerebrospinal fluid (CSF) biomarkers of tau-pathology and Aβ are established in AD diagnostics. The same markers have been reported to associate with DLB in a small study (Irwin et al., 2018). However, the associations seem to largely stem from concomitant AD-pathology, albeit Aβ-42 levels were slightly lower also in DLB with low AD co-pathology compared to controls. This difference might be attributed to two outliers that had a large impact on the mean Aβ-42 level as the group consisted of only 14 individuals.

A diagnosis of DLB is probable when there are at least two core clinical features or one core clinical feature with at least one biomarker and dementia begins before or at the same as parkinsonism (Yamada et al., 2019). The diagnostic criteria are summarized in Table 2.

Neuropathological examination still represents the golden standard in DLB diagnostics. In neuropathological assessment, LRP can be divided into five categories: diffuse neocortical, limbic, brainstem-predominant, amygdala-predominant, olfactory bulb only, which together with the National Institute on Aging – Alzheimer‘s Association guidelines (NIA-AA) assess the likelihood of typical DLB (McKeith, I. G. et al., 2017). To date, no large-scale analyses have

been performed to assess if the LRP category affects the clinical picture have been conducted. Besides LRP, AD co-pathology is present in 50-80% of DLB cases (Robinson et al., 2018).

Table 2: The diagnostic criteria of DLB according to the DLB consortium. The criteria are divided into main criteria and supportive criteria.

Core clinical features 1. Fluctuating cognition 2. Recurrent visual hallucinations (complex and well-formed) 3. REM sleep behavior disorder 4. ≥1 main features of parkinsonism: bradykinesia, resting tremor, rigidity
Indicative biomarkers 1. Reduced dopamine transporter uptake in basal ganglia seen in SPECT/PET 2. Low uptake of 123I-MIBG in myocardial scintigraphy 3. REM sleep without atonia
Probable DLB: Dementia AND 1. ≥2 core clinical features with/without indicative biomarkers OR 2. 1 core clinical feature + ≥1 indicative biomarker(s)

There is no cure for DLB with the effects of current treatments limited and affecting symptoms and not disease advancement per se. Exercise and cognitive training are thought to be beneficial and can improve quality of life. No DLB-specific drugs are in routine use, rather, there is overlap in the pharmacological treatment of DLB, AD and PD. Cholinesterase inhibitors (donepezil and rivastigmine) are the first-line of drugs (Taylor et al., 2019) for both cognitive and psychiatric symptoms. Memantine can alleviate cognitive symptoms and parkinsonism may respond to careful treatment with levodopa. The use of antipsychotics and antidepressants for neuropsychiatric symptoms should only be used after individual consideration (McKeith, I. G. et al., 2017; Taylor et al., 2019).

2.4 Amyotrophic lateral sclerosis

2.4.1. Definition and epidemiology

ALS is a neurodegenerative disease where the upper and lower motor neurons in the brain and spinal cord gradually die causing the loss of voluntary movements, which eventually leads to respiratory failure. In ALS with FTLD, neurons also in the frontal and temporal lobes are affected. Up to a half of ALS patients develop at least mild cognitive or psychiatric symptoms and more than one in ten fulfills the criteria for the behavioral variant of frontotemporal dementia (Crockford et al., 2018; Phukan et al., 2012). Despite their clinical differences, ALS and FTLD share many common pathologic and genetic features.

ALS is a relatively rare disease, its median prevalence has been estimated to be 5.4 per 100 000 in Europe, 3.4 in North America, 2.01 in China and the highest in Japan with prevalence of 11.4 per 100 000 (Chio et al., 2013). In 1976-81 the prevalence was 6.4 in Middle-Finland (Murros & Fogelholm, 1983) but there are no more recent studies. Based on the annual incidence of ca. 150-200 cases, median survival of three years and the proportion of 5-10% of cases with slowly progressive disease, the number of ALS patients in Finland is estimated to be 400-700 (Hannu Laaksovirta, personal communication) placing Finland in the upper category of ALS prevalence. The incidence of ALS is greatest in the age group of 60-75 years, but familial ALS especially can begin considerably earlier.

Approximately 5-10% of ALS is familial (fALS) (Byrne et al., 2011) and caused by a pathogenic variant, most often the hexanucleotide repeat expansion in *C9orf72* or in the *SOD1* gene *(Andersen, 2006; DeJesus-Hernandez et al., 2011; Renton et al., 2011)*. The mechanisms behind sporadic ALS (sALS) are more complex and environmental factors are suspected to play a role as well (Ingre, Roos, Piehl, Kamel, & Fang, 2015), thus the cause of the disease often remains unknown. ALS has been modelled to be a multistep process and these steps can be genetic or environmental (Al-Chalabi et al., 2014). Many environmental factors have been proposed to increase the risk of ALS but only smoking has proven consistent as a risk factor for ALS (Armon, 2009). A recent Mendelian randomization study also suggested hyperlipidemia as a risk factor for ALS and light physical activity as a protective factor (Bandres-Ciga et al., 2019). Immune system dysfunction is also suspected in ALS, supported by observations that autoimmune diseases such as asthma, celiac disease and systemic lupus erythematosus are more common in ALS patients than in the general population (Turner, Goldacre, Ramagopalan, Talbot, & Goldacre, 2013).

2.4.2. Classification and clinical picture

ALS can start focally with any voluntary muscle, so early clinical pictures vary and clinical diagnosis can be challenging. Diagnosis is based on clinical symptoms, electroneuromyography and ruling out other possible causes. Differential diagnoses include

peripheral neuropathy, radiculopathy, borreliosis, vitamin B12 deficiency, thyroid dysfunction, and toxic exposures, upper motor neuron diseases (HSP, PLS) and spinal muscular atrophy subtypes. MRI is often used but its primary function is to rule out other potential causes since in less than half of ALS cases brain MRI shows abnormal signals at the corticospinal tract and results correlate poorly with clinical data (Hecht et al., 2001).

To effectively summarize the possible clinical pictures, ALS is often grouped by different criteria. First, ALS can be sporadic or familial. This dichotomy seems simple enough, but e.g. *SOD1* mutations can have reduced penetrance and within the same family with the same mutation, the clinical picture and age of disease-onset can vary greatly (Rezania et al., 2003). Another classification that is often used is the clinical presentation at the time of diagnosis (spinal onset - bulbar onset – limb-onset, symmetric - asymmetric) (Al-Chalabi et al., 2016). In as heterogeneous a disease as ALS, classification of patients is important for several reasons. First, there is a need for clear criteria of clinical diagnoses so that patients are treated appropriately and that the relevant and right counseling for likely disease progression can be given. Second, different clinical pictures can represent different subtypes of the disease that might respond differently to treatments. Third, different information is useful in clinical trials where stratification with e.g. genotype information can change results. This is especially true for the current gene-based clinical trials emerging in ALS (clinicaltrials.gov, identifiers NCT04220021 (*C9orf72)* and NCT02623699 (*SOD1*)*)*.

2.4.3 ALS genetics

The first ALS gene, *SOD1*, was discovered in 1993 (Rosen et al., 1993). *SOD1* encodes the superoxidase dismutase 1 protein that functions against oxidative stress. Dozens of mutations in *SOD1* have been reported and no one key pathological mechanism has been found, although the major mechanism is thought to be accumulation of misfolded proteins (Andersen, 2006). *SOD1* ALS differs from other types of ALS in that it lacks TDP-43 or FUS pathology (Renton, Chio, & Traynor, 2014). Even though mutations are in the same gene, there is marked variability between patients with different *SOD1* mutations. For example, *SOD1* p.A4V, which is the most common *SOD1* mutation in the USA, leads to a rapidly progressing form of ALS that usually results in death within a year or two of symptom onset (Marangi & Traynor, 2015). However, the *SOD1* p.D91A (formerly denoted D90A) mutation that is the most common *SOD1* mutation in Finland and Scandinavia, causes a slowly progressive form of ALS mostly in people homozygous for the mutation. The clinical picture is usually well recognizable and starts with a stiffness in the lower limbs that turns into paresis that progresses over many years (Andersen, 2006).

To date more than 30 genes have been associated with ALS (Maurel et al., 2018). In Caucasian populations they explain roughly 70% of familial and 15% of sporadic cases (Chia, Chio, & Traynor, 2018) (Figure 7). A major breakthrough in ALS genetics came in 2011 when the *C9orf72* hexanucleotide (GGGGCC) repeat expansion was identified (DeJesus-Hernandez et al., 2011; Renton et al., 2011) underlying the clear GWAS association of chromosome 9p21

locus (Laaksovirta et al., 2010) with ALS. The *C9orf72* hexanucleotide repeat expansion is the most common cause of both sporadic and familial ALS in European populations (Majounie et al., 2012). In an analysis of European cases, 8% of sporadic ALS and 40% of familial ALS were caused by the *C9orf72* hexanucleotide repeat expansion. Finland is among the countries with the highest *C9rof72* expansion frequencies and here 21.1% of sporadic and 46% of familial ALS were caused by the expansion (Majounie et al., 2012)

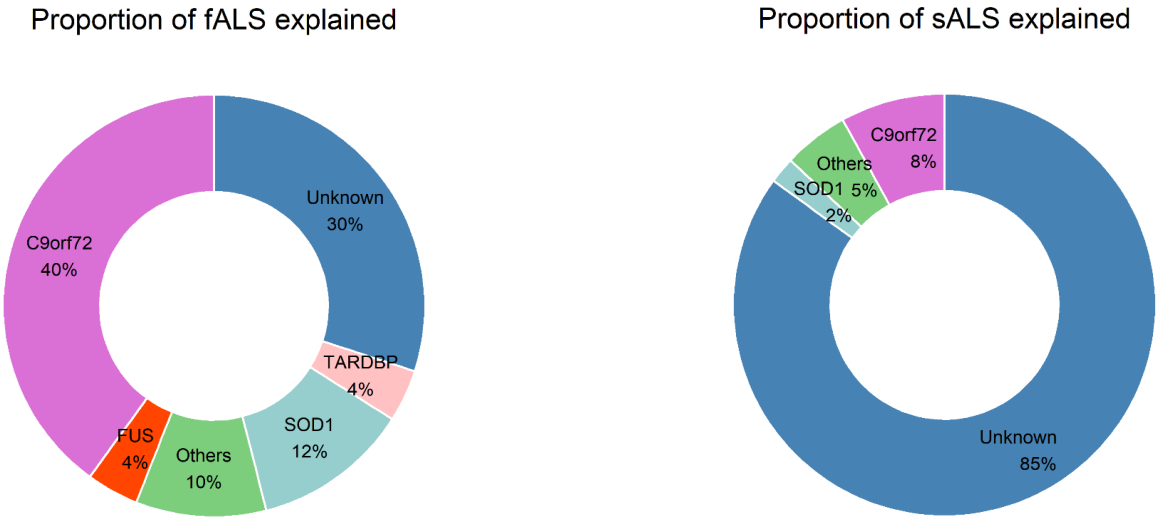

Figure 7: Percentage of familial ALS (fALS) and sporadic ALS (sALS) explained by genetics in Caucasian population (Chia et al., 2018). For most fALS cases, a causative genetic variant can be detected. The opposite is true for sALS demonstrating its complexity as a disease. In Finland, ~ 21% of sporadic and ~46% of familial ALS are caused by the *C9orf72* expansion (Majounie et al., 2012).

2.4.4. *C9orf72*

The identification of *C9orf72* GGGGCC hexanucleotide repeat expansion as a cause of ALS was challenging as it could not easily be captured by routine variant-calling software and was hard to identify from aligned sequences (Figure 8). Its identification was a hallmark in ALS genetics since it was revealed to be the most common genetic cause of ALS in populations of European descent and also highlighted the importance of RNA metabolism in ALS. After its association with ALS and FTLD was found, there were many studies investigating whether the *C9orf72* expansion also caused other neurodegenerative diseases. There have been several articles on the association of the expansion with AD, but their results have been reviewed to

be mixed (Liu, Y., Yu, Zong, Zhou, & Tan, 2014) and confounded by the potential misdiagnosis of AD in FTLD cases and issues with statistical power. However, it seems quite certain that the expansion is not a major cause of AD since in AD studies, its prevalence was less than 1% (Kohli et al., 2013a; Majounie et al., 2012) and the *C9orf72* locus has not been detected in the large AD GWASes (Jansen et al., 2019; Kunkle et al., 2019). The association of PD with the expansions has also been studied since parkinsonism is seen in up to a third of FTLD patients (Liu, Y. et al., 2014). The results were largely the same as in AD: it was reviewed that individual studies identified the expansion in less than 1% of PD but in the majority of studies, no expansion carriers in PD were found (Bourinaris & Houlden, 2018). Again, the number of cases in studies with no expansions identified were typically under a few hundred. Nevertheless, the expansion is not a common cause of PD.

It is still an open question as to what repeat length is pathogenic and classifiable as an expansion. In the original article, repeat length of 30 was used (Renton et al., 2011) as the expansion threshold. It is important to note, that this threshold was selected based on methodological limitations rather than any biological criteria. To date, there is no consensus on pathogenic repeat length, so the original threshold of 30 is often still used. There have been studies examining the effect of "intermediate" repeat lengths on both ALS/FTLD and other neurodegenerative diseases. This effort is complicated by the fact that like with expansion, there is no standard length for "intermediate" allele length. Often ≥7 repeats is the threshold for intermediate repeats since those repeat lengths associate with the same haplotype as expansions (van der Zee et al., 2013). It was recently reviewed that intermediate repeats do not predispose to a variety of neurodegenerative diseases but might contribute to psychiatric symptoms (Ng & Tan, 2017). Subsequently, new studies observing significant effects of intermediate alleles have been published. A new meta-analysis that supplemented a previous meta-analysis (Chen et al., 2016) with a British/Alzheimer's Disease Neuroimaging Initiative dataset had a total of 5071 cases and 3747 controls and found an association between ALS and intermediate C9orf72 repeats of 24-30 repeats (OR=4.20, p=0.02 random-effects test) (Iacoangeli et al., 2019). Another recently published study observed that intermediate length alleles of ≥ 17 repeats associated with corticobasal degeneration. They then proceeded to *in vitro* studies and showed that these alleles increased *C9orf72* expression in human brain and in neural progenitor cells (Cali et al., 2019).

Despite having been relatively recently discovered, major progress in understanding the pathological mechanisms of *C9orf72* expansions has been made. Three main mechanisms have been identified: 1. loss-of-function of C9orf72, 2. toxic gain-of-function due to repeat RNA (sense and antisense) and 3. toxic gain-of-function of dipeptide repeat proteins translated from expansion RNA (Figure 9).

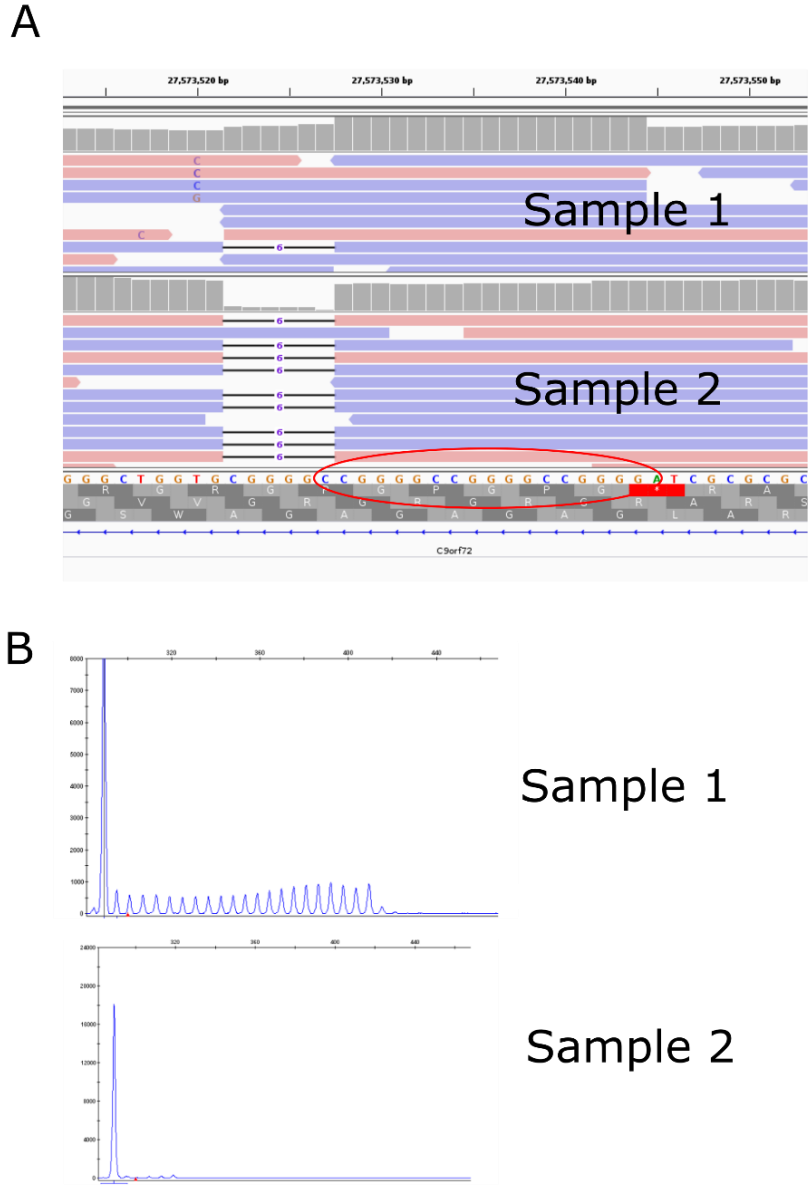

Figure 8: A. *C9orf72* hexanucleotide GGGGCC repeat in a genomic context. The repeat length cannot be determined on routine alignment since standard alignment software cannot map repeat containing reads well. B. Assessing repeat length with repeat-primed PCR and capillary electrophoresis. The repeat length of Sample 1 is 2/24 and Sample 2 is 2/2.

Loss-of-function toxicity could be due to deficits in several cellular trafficking pathways that include inhibited autophagy, reduced endocytosis and impaired lysosomal function. *C9orf72* knock-out cells are also susceptible to excitotoxicity due to surplus glutamate receptors (Selvaraj et al., 2018). The function of *C9orf72* in immune regulation also seems to be important (Balendra & Isaacs, 2018). Importantly, *C9orf72* knock-out mice do not develop motor neuron degeneration suggesting that loss-of-function of C9orf72 is probably not a major component of pathogenic mechanisms. However, the knock-out mice develop a fatal autoimmune disease that has some similarities to systemic lupus erythematosus (Burberry et al., 2016). A recent study on mice with loss of C9orf72 from myeloid cells alone caused the same autoinflammatory phenotype and it was linked to impaired degradation in the stimulator of interferon genes (STING) protein via the autolysosomal pathway (McCauley et al., 2020). STING has been reviewed to be an important inducer of autoimmune diseases including systemic lupus erythematosus (Kumar, 2019). The C9orf72 knock-out myeloid cells' oversensitivity to STING can explain the surplus of autoimmune diseases in ALS patients (Turner et al., 2013).

Toxic gain-of-function mechanisms include RNA secondary structures such as G-quadruplexes that lead to abnormal binding and depletion of many RNA-binding proteins (DeJesus-Hernandez et al., 2011; Donnelly et al., 2013). RNA-binding proteins are important in RNA regulation and act in splicing, degradation and transport of RNA. The role of dipeptide repeat proteins is more unclear since in post-mortem studies dipeptide repeat proteins do not overlap well with TDP-43 pathology and are present in CNS regions not affected by ALS/FTLD. Nevertheless, dipeptide repeat proteins may be important in earlier stages of disease and there is evidence of its neurotoxicity in disease models (Balendra & Isaacs, 2018). Furthermore, the accumulation of dipeptide repeat proteins may have a synergetic effect with the loss-of-function of C9orf72 that inhibits autophagy and the clearance of dipeptide repeat proteins (Boivin et al., 2020).

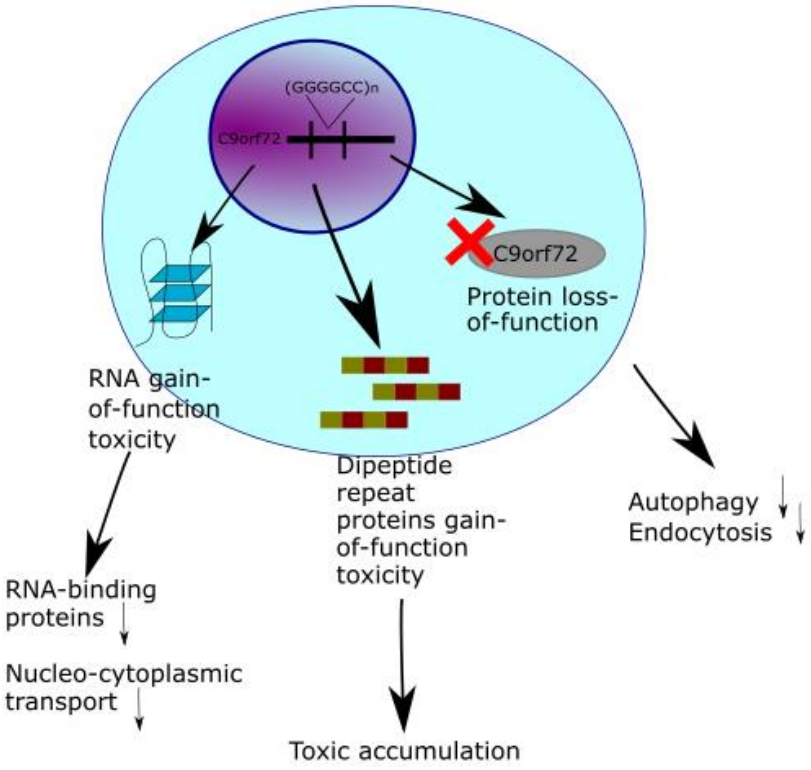

Figure 9. Key molecular mechanisms of the *C9orf72* hexanucleotide repeat expansion.

2.4.5. Molecular mechanisms and neuropathologic features

The molecular mechanisms behind ALS vary with the underlying cause but the common pathway is aggregation of ubiquitinated proteins that can be seen as cytoplasmic inclusions in motor neurons but also in cerebellar cells in the CNS (Al-Chalabi et al., 2012). TAR DNA-binding protein 43 (TDP-43) is a major component of these inclusions (Arai et al., 2006; Neumann et al., 2006). Even in patients with no *TARDBP* mutations, TDP-43 is present in the vast majority of SOD1-negative ALS patients and pathological inclusions positive for TDP-43-immunohistochemistry are seen in motor neurons of the anterior columns of the spinal cord the neurons of hypoglossal nuclei, and possibly in neurons of the primary motor cortex, hippocampus and frontal cortex. In addition, TDP-43-positive neurites are often seen in these areas (Blokhuis, Groen, Koppers, van den Berg, L H, & Pasterkamp, 2013). Especially toxic are

TDP-43 C-terminal fragments that are prone to aggregation. In rat studies, the aggregation tendency of mutant TDP-43 is often greater but it seems that both mutant and wild-type TDP-43 can be neurotoxic and increased levels is the key to its pathogenicity (Barmada et al., 2010). In ALS patients with mutations in *FUS*, there are FUS-positive cytoplasmic inclusions. Like TDP-43, *FUS* is an RNA-binding protein but whether its aggregates are consistently present in ALS patients without FUS-mutations is unclear since both findings are reported (Blokhuis et al., 2013). Also, some *SOD1* mutations lead to accumulation of SOD1 inclusions, but inclusions of misfolded wild type SOD1 have also been reported in ALS patients with mutations in other ALS genes than *SOD1,* which suggests that misfolding also of wild type SOD1 can be part of the pathogenic pathway (Forsberg et al., 2019).

Similar to α-synuclein in DLB, SOD1, FUS and TDP-43 proteins have been hypothesized to have prion-like qualities that would allow them to spread from cell to cell. It has been observed especially with SOD1 that misfolded proteins seen in ALS could cause their native counterparts to aggregate. The hypothesis is that protein destabilizing mutations in *SOD1* (and *FUS* gene and C-terminal region of *TARDBP)* cause prion-like domains to be unfolded that then work as seeds for further aggregation initiating a self-perpetuating cycle (Polymenidou & Cleveland, 2011). In cell culture, these protein aggregates have been shown to spread from cell to cell even without direct contact via micropinocytosis and this transfer seems efficient (Munch, O'Brien, & Bertolotti, 2011). Since then, this spreading has been demonstrated also *in vivo* in mice where the injection of mutant SOD1 protein into the sciatic nerve triggered a spreading motor neuron disease (Ayers, Fromholt, O'Neal, Diamond, & Borchelt, 2016).

Other pathological mechanisms suggested in ALS include oxidative stress, mitochondrial dysfunction, axonal damage, excitotoxicity and neuroinflammation. The role of oxidative stress is highlighted by the normal function of SOD1: it has been reviewed to clear reactive oxygen species, molecules that can damage e.g. DNA, proteins and lipids (Tarafdar & Pula, 2018). Mitochondria are the prime location of energy production in cells. In mitochondria, oxidative phosphorylation creates ATP but also reactive oxygen species as a by-product. Thus, mitochondria are prone to oxidative damage especially when there is additional cell stress. Coupled with neurons' high metabolism and resulting oxygen use, neurons are regarded to be highly susceptible to oxidative stress and oxidative damage (Singh, Kukreti, Saso, & Kukreti, 2019). Therefore, it is no surprise that oxidative stress is associated with several neurodegenerative diseases like AD and PD, and not just ALS. In ALS, excess reactive oxygen species and resulting damaged molecules have been found in blood, urine and cerebrospinal fluid (Liu, J. & Wang, 2017).

Excitotoxicity is a phenomenon where nerve cells are damaged and die due to overactivation of receptors of excitatory amino acids (most often glutamate) which leads to an influx of ions, particularly calcium (Dong, Wang, & Qin, 2009). The *C9orf72* hexanucleotide repeat containing RNA has been shown to bind the glutamate receptor (GluR2) sequestering enzyme ADARB2 and thus preventing normal editing of the receptor pre-mRNA (Donnelly et al., 2013). This in turn leads to sensitivity for glutamate excitotoxicity in cultured iPS-neurons from ALS patients with the *C9orf72* repeat expansion. Clinically, this mechanism is supported

by abnormal glutamate metabolism in ALS (Plaitakis & Caroscio, 1987) and the (albeit modest) effect of riluzole that modulates the glutamatergic system in ALS (Bensimon, Lacomblez, & Meininger, 1994).

In addition to motor neurons, glial cells may also affect the pathogenesis of ALS. Microglial activation is seen in ALS and microglia are viewed to have an important role in maintaining a suitable environment for nerve cells via e.g. uptake of misfolded proteins (Liu, J. & Wang, 2017). The role of astrocytes in the pathogenesis of ALS has also been recently studied. It has been reported that astrocytes from both fALS and sALS patients are toxic to motor neurons (Haidet-Phillips et al., 2011). Interestingly, astrocytes differentiated from fibroblasts of a sALS patient, an ALS patient with *C9orf72* hexanucleotide expansion, and an ALS patient with *SOD1* A4V mutation were all toxic toward motor neurons (Meyer et al., 2014). This observation suggests that changes in astrocyte function may be a common mechanism in ALS. The potential role of glial cells in the pathogenesis of ALS highlights the importance of understanding the environment and the interaction between cell types.

2.4.6. Treatment

At the moment, riluzole is the most widely used drug for ALS and prolongs survival, albeit on average by only three months (Bensimon et al., 1994). Patients generally survive only 2-5 years after diagnosis (Miller, Mitchell, & Moore, 2012). Even though present treatment options are scarce and have a limited effect, increasing understanding of genetic and pathological mechanisms behind ALS promises treatments that are more effective. At the moment, there are dozens of ongoing clinical trials (https://clinicaltrials.gov/, accessed 14.7.2020) and encouraging results from antisense oligonucleotide (ASO) therapies have raised interest as a future treatment (Klim, Vance, & Scotter, 2019). ASOs are a versatile tool: they bind to mRNA in the nucleus and can promote its cleaving, effectively silencing a gene. They can also inhibit translation in ribosomes or alter splicing. Nusinersen, a drug for spinal muscular atrophy, increases SMN1 expression by converting SMN2 mRNA into SMN1-like mRNA. Albeit effective (Finkel et al., 2017), the very high price of Nusinersen (and most likely future ASOs) restricts its use and has sparked major debate on whether the drug is given and to whom in public hospitals.

2.5. Alzheimer's disease

2.5.1. Definition and epidemiology

AD is the most common cause of dementia (Ince et al., 1995) and arguably the best-known neurodegenerative disease in the general population. Its hallmark is progressive loss of memory. AD can be familial (early-onset) or sporadic (late-onset). The prevalence of AD is affected by age and whether clinical or neuropathological diagnosis is used. In different autopsy series focusing on patients with dementia, the frequency of AD ranged from 64% to 90% (Barker et al., 2002). In a population-based study of individuals over 75 years in the Kuopio region in Finland, the prevalence of AD was 10.6%, which accounted for 47% of all demented individuals (Rahkonen et al., 2003). However, the main neuropathological changes associated with AD, neuritic plaques and neurofibrillary tangles (NFTs), are even more prevalent than the actual disease. Even in individuals with generally minimal neuropathological changes, there were neuritic plaques in 50% and tau pathology in 93% of cases (mean age 70) (Robinson et al., 2018).

2.5.2. Clinical picture

The stage of AD defines its clinical picture. In early-stage (mild) AD, memory loss and other cognitive dysfunctions are not yet necessarily evident and can include difficulties in remembering recently read material or misplacing objects. In the middle-stage (moderate) AD, patients require external help in their daily lives. Symptoms include evident forgetfulness, confusion, and behavioral changes. In the late-stage (severe) AD, patients require continuous assistance, awareness of surroundings is lost, communication becomes difficult and physical abilities greatly weaken.

Probable AD dementia is diagnosed clinically when there is dementia and 1. the onset was gradual over months to years, 2. the cognitive functions have clearly worsened over time and 3. the initial and most prominent deficits are amnestic (especially impairment in learning and recall of recently learned information) with dysfunction in other non-amnestic cognitive processes such as impaired executive dysfunction, impaired visuospatial abilities, impaired language functions or changes in personality or behavior. The most prominent deficit in AD can also be non-amnestic but in those cases there should also be deficits in other cognitive domains (McKhann et al., 2011). The golden standard of AD diagnosis is neuropathological examination. The likelihood of AD is high if there are frequent neuritic plaques in the neocortex according to the Consortium to Establish a Registry for Alzheimer Disease (CERAD) criteria and neurofibrillary pathology corresponding to Braak stage V/VI.

2.5.3. AD genetics

The genetics of AD vary between familial and sporadic forms. In familial AD, the disease onset is usually before 65 years and there is a pathogenic mutation in *APP (Goate et al., 1991)* on chromosome 21, in *PSEN1 (Sherrington et al., 1995)* in chromosome 14 or in *PSEN2 (Levy-Lahad et al., 1995)* on chromosome 1. All three of these genes are involved in Aβ metabolism. *APP* encodes amyloid precursor protein and *PSEN1* and *PSEN2* encode presenilins 1 and 2, which regulate APP processing via the γ-secretase enzyme. A recent review counted over 50 APP mutations, more than 30 in *PSEN2* and more than 200 in *PSEN1* that have been identified in AD but together they explain only ca. 10% of familial AD cases *(Cacace, Sleegers, & Van Broeckhoven, 2016)*.

The sporadic or late-onset AD is a complex disease and has many genetic risk factors. *APOE* ε4 is the most common genetic risk factor (Strittmatter et al., 1993). Its mean frequency in populations under 65 years is ca. 15% (Eisenberg, Kuzawa, & Hayes, 2010) and it has an odds ratio of 3-15 depending on the number of copies carried (Amouyel, Brousseau, Fruchart, & Dallongeville, 1993).

In recent years, two exceptionally large meta-analysis GWASes on AD have been published with tens of thousands of cases and controls (Jansen et al., 2019; Kunkle et al., 2019). In both, the *APOE* locus was the strongest association signal. Besides *APOE,* the studies reported 20 (Kunkle et al., 2019) and 28 (Jansen et al., 2019) loci with genome-wide significant associations (Table 3). These loci largely overlap with those reported in previous GWASes but some previously reported loci did not reach genome-wide significance. Variants in the genes *ABCG1*, *GALNT7, MEF2C, NME8, GAB2, EXOC3L2, TRIP4* and an intergenic region in chromosome 9 were not replicated in these two large meta-analysis GWASes by Beecham et. al (Beecham et al., 2014; Ruiz et al., 2014; Seshadri et al., 2010),

Table 3. Loci with genome-wide significant associations in AD in two recent large meta-analysis GWAS projects.

Chromosome	Gene/Locus	Reported by Jansen et al.	Reported by Kunkle et al.
1	*ADAMTS4*	X	
1	*CR1*	X	X
2	*BIN1*	X	X
2	*INPPD5*	X	X
3	*HESX1*	X	
4	*CLNK*	X	
6	*HLA-DRB1*	X	X
6	*TREM2*	X	X
6	*CD2AP*	X	X
7	*ZCWPW1*	X	X (NYAP1)
7	*EPHA1*	X	X
7	*CNTNAP2*	X	
8	*CLU/PTK2B*	X	X
10	*ECHDC3*	X	X
11	*SPI1 (CELF1)*		X
11	*MS4A locus*	X	X
11	*PICALM*	X	X
11	*SORL1*	X	X
14	*FERMT2*		X
15	*ADAM10*	X	
15	*APH1B*	X	
16	*KAT8*	X	
17	*SCIMP*	X	
17	*ABI3*	X	
17	*ACE*		X
18	*ALPK2*	X	
19	*ABCA7*	X	X
19	*APOE*	X	X
19	*AC074212.3*	X	
19	*CD33*	X	X
20	*CASS4*	X	X

2.5.4. Molecular mechanisms and neuropathology

AD can be thought to develop through two stages. First is a preclinical stage with neuropathological changes but no apparent cognitive symptoms (Price & Morris, 1999). In the second stage there is mild cognitive impairment. However, not all individuals with preclinical

neuropathological changes progress to mild cognitive impairment and not all who have mild cognitive impairment develop dementia (Petersen et al., 1995; Varatharajah, Ramanan, Iyer, Vemuri, & Alzheimer's Disease Neuroimaging Initiative, 2019).

In the preclinical stage, there are neuritic plaques (extracellular Aβ accumulations) and NFTs in the entorhinal cortex and hippocampus CA1 region and there is none to little cell loss in these regions. In comparison, in "normal" aging there are fewer neuritic plaques, and entorhinal and hippocampal regions have fewer NFTs and there is no cell loss (Price, Davis, Morris, & White, 1991; Price & Morris, 1999). Modern imaging technologies includindg high PET amyloid tracer retention and cortical thinning/hippocampal atrophy on structural MRI with CSF Aβ and tau markers have been proposed as biomarkers for preclinical AD in research use (Sperling et al., 2011).

According to NIA-RI criteria, the neuropathological diagnosis of AD requires that there is frequent and widespread neuritic plaques and NFTs (Consensus recommendations for the postmortem diagnosis of alzheimer's disease. the national institute on aging, and reagan institute working group on diagnostic criteria for the neuropathological assessment of alzheimer's disease.1997). The NIA-RI criteria are based on two classification schemes: CERAD (Mirra et al., 1991b) and Braak stage (Braak, Alafuzoff, Arzberger, Kretzschmar, & Del Tredici, 2006; Braak & Braak, 1995). CERAD focuses on the frequency of neuritic plaques whereas Braak staging focuses on NFTs.

The neuropathological criteria for AD highlight the importance of neuritic plaques and NFTs in AD. The main component of NFTs is hyperphosphorylated tau that aggregates into fibrils. Tau normally associates with microtubules (Witman, Cleveland, Weingarten, & Kirschner, 1976) but its phosphorylation induces its release from microtubules, which allows it to aggregate (Biernat et al., 1992). According to NFT Braak staging in AD, NFTs first appear in the transentorhinal and the entorhinal regions (stages 1 and 2), then proceed to the limbic regions including hippocampus CA-1 (stage III). From there, NFTs become more widespread until at last (stage VI), there is widespread neocortical NFT pathology (Braak & Braak, 1995). The other pathological hallmark of AD is neuritic plaques whose main component is Aβ. Different Aβ peptides are cleaved by β and γ-secretases from APP. Neuritic plaques are extracellular aggregations of amyloid fibrils (Glenner & Wong, 1984) but Aβ is not only observed as parenchymal deposits in AD. It is also present as cerebrovascular changes (in small arterioles and capillaries) in cerebral amyloid angiopathy (CAA) (Pantelakis, 1954). CAA is not specific to AD and is observed in 20-60% of non-demented individuals, in 50-80% of demented individuals and in approximately 90% of AD patients (Arvanitakis et al., 2011; Keage et al., 2009; Mandybur, 1975). CAA can be divided into two types. In type 1, there is Aβ in at least cortical capillaries and in type 2 there are amyloid deposits in cortical and leptomeningeal arteries but not in capillaries (Attems, Jellinger, Thal, & Van Nostrand, 2011; Attems, Yamaguchi, Saido, & Thal, 2010).

The Aβ cascade is a key pathway in the development of familial AD as demonstrated by the three genes (*APP, PSEN1, PSEN2*) whose mutations lead to familial AD. In sporadic AD, Aβ processing is regarded as an important pathway, but other factors are crucial as well. *APOE* is important in lipid metabolism but it also affects immune responses, these have been defined

especially in innate immune cells and brain microglia (Huebbe & Rimbach, 2017; Krasemann et al., 2017). Another important gene that highlights the importance of immune responses in AD is *TREM2*. The rare (MAF 0.25% in Europe) R47H substitution has an effect size comparable to *APOE ε4* (Jonsson et al., 2013). The two new large GWASes strengthen the importance of immunological pathways as e.g. *HLA-DRB1, CR1* and *CD33* were associated with AD in both studies. Variants in *MAPT,* the gene that encodes tau, have not associated with AD in the recent GWASes but tau binding proteins were implicated in pathway analyses. In other studies, the H2 MAPT haplotype has been associated with a reduced risk of AD (Allen et al., 2014) (Figure 10).

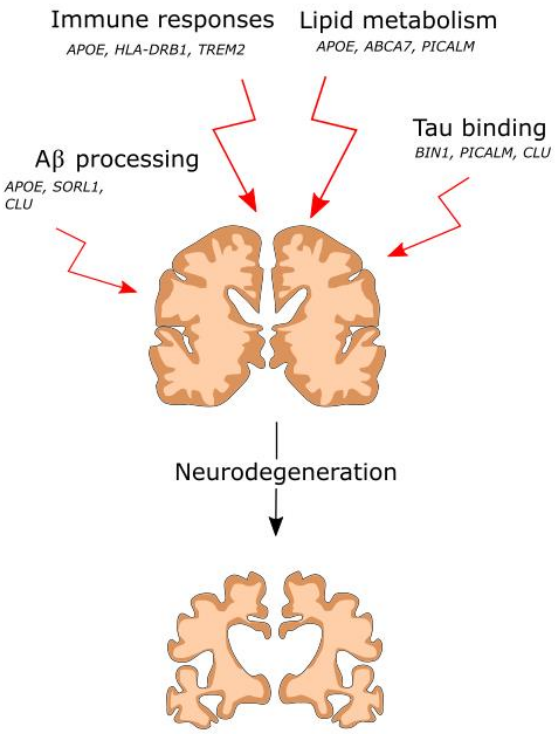

Figure 10. Main molecular pathways of late-onset AD and examples of genes in pathways. Different cellular processes lead to neurodegeneration and visible loss of brain tissue. Genetic variations in one gene can play a role in multiple pathways, e.g. APOE is implicated in Aβ processing, immune responses and lipid metabolism.

2.5.5. Treatment

There is no disease-modifying treatment for AD. The prominence of Aβ pathology in AD and the amyloid cascade hypothesis as the primary mechanism behind AD has led to large efforts in immunotherapies targeting the amyloid protein. However, to date no clinical trial has been successful either due to lack of effect or severe side effects.

The most used pharmacological treatments include cholinesterase inhibitors and memantine. Cholinesterase inhibitors ease symptoms in early and mid-phases of AD leading to better cognitive function and improved quality of life (Rogers & Friedhoff, 1996). In AD, cholinergic neurons and neural circuits deteriorate (Bartus, Dean, Beer, & Lippa, 1982). Cholinesterase inhibitors increase acetylcholine concentration, which improves neurotransmission and alleviates symptoms.

Memantine is another widely used drug for AD, albeit it seems to alleviate symptoms in only moderate to severe AD (Schneider, Dagerman, Higgins, & McShane, 2011). Memantine blocks NMDA receptors (glutamate receptors) and decreases excitotoxicity (Kornhuber, Weller, Schoppmeyer, & Riederer, 1994).

3. AIMS OF THE STUDY

The general aim of this study was to investigate different genetic variations behind ALS, AD and DLB.

The specific aims were:

1. Publication I: To study if *C9orf72* hexanucleotide repeat intermediate length alleles are a risk factor for AD or cognitive impairment. The second objective was to describe the *C9orf72* allele length distribution in an older Finnish population to assess the pathogenic repeat length.

2. Publication II: To genotype a large number of Finnish individuals with ALS and controls in order to study the potential effects of *C9orf72* hexanucleotide intermediate alleles on ALS and their clinical relevancy.

3. Publication III: To study the distribution of LRP in the central nervous system in an aged population-based cohort and its association with AD pathology.

4. Publication IV: To study how the previously reported AD risk loci associate with the different neuropathological features of AD (CERAD score, Braak staging, cerebral amyloid angiopathy and capillary Aβ).

4. MATERIALS AND METHODS

4.1. Study cohorts

4.1.1. Vantaa85+ study

The Vantaa 85+ Study consists of individuals of at least 85 years of age, who were living in the city of Vantaa, Finland on April 1st 1991. Professor Raimo Sulkava and chief physician Leena Niinistö initiated the study. There were 601 eligible individuals and of them, 553 participants underwent neurological examination including MMSE by two neurologists in 1991–1992, with follow-up studies in 1994, 1996, 1999, and 2001. Neuropathological autopsy was performed on 306 individuals.

Neuropathological examinations included assessment of α-synuclein with immunohistochemistry using a mouse monoclonal anti-α-synuclein antibody (clone 5G4, 1:1000, AJ Roboscreen GmbH, Leipzig, Germany or Merck KGaA, Darmstadt, Germany) (Kovacs et al., 2012). α-synuclein was assessed from 11 anatomic regions: sacral and thoracic spinal cord, medulla oblongata, pons, midbrain, amygdala, hippocampus from the right hemisphere, cingulate cortex, frontal cortex, temporal cortex and parietal cortex. This allowed semiquantitative scoring of LRP and the classification of individuals.

Neuritic plaques were assessed by Bielshowsky silver stain according to CERAD protocol (Mirra et al., 1991a) and neurofibrillary tangles by Gallyas silver stain method according to the protocol by Braak and Braak (Braak & Braak, 1995)

4.1.2. Helsinki Birth cohort study

The Helsinki Birth cohort study consists of 8760 individuals born in Helsinki in 1934-1944 (Barker et al., 2005, Eriksson et al., 2006). A random subsample of 2003 individuals underwent DNA extraction (Kajantie et al., 2012, Yliharsila et al., 2007) and assessment of cognition with MMSE and CERAD in 2001-2004. In addition, information on cognition and psychiatric diagnoses were available up till December 31 of 2013. These data included dementia hospitalizations and dementia deaths as well as psychiatric diagnoses (F00-F99 for ICD-10, 290-319 for ICD-9 and 290-299, 301 for ICD-8) and selected neurodegenerative diseases such as AD, Parkinson disease, ALS/FTLD and multiple sclerosis (G10-13, G20-26, G30, G35-37 for ICD-10, 331-333 and 340-341 for ICD-9, 340-341 and 348 for ICD-8) . These data were obtained from the Finnish Hospital Discharge and Causes of Death Registers.

4.1.3. Helsinki Businessmen Study

Helsinki Businessmen Study participants were originally 3,490 healthy Finnish businessmen or executives, born 1919-1934 with similar high socioeconomic status. In 2002-2003, 672 home-living but otherwise randomly selected men were selected as a subsample and their cognition tested using MMSE (Strandberg et al., 2016) with 650 also giving a venous blood sample from which DNA was extracted. In 2014, their cognition was re-evaluated with a questionnaire.

4.1.4. DEBATE study

The DEBATE cohort is based on a random sample of 4,800 individuals who were living in Helsinki, Finland. The studied subsample was formed in 2000 from 400 home-living individuals with stable cardiovascular disease (peripheral artery disease, coronary artery disease, stroke or transient ischemic attack). The individuals in the subsample were clinically examined and their cognitive function were assessed with MMSE (Uusvaara, Pitkala, Kautiainen, Tilvis, & Strandberg, 2013). Additionally in 2014, dementia diagnoses were screened from death certificates.

4.1.5. ALS patients

The ALS cohort consisted of 773 ALS patients who were recruited at the Department of Neurology, Helsinki University Hospital, since 1994. The department receives referrals from neurologists throughout Finland. There were 177 (23%) familial ALS cases and 596 apparently sporadic cases. The patients' mean age-of-onset was 58 years and 51% were females. The *C9orf72* repeat expansion was in 25% of all ALS cases.

4.1.6. PLASTICITY study

The PLASTICITY study (Hokkanen, Launes, & Michelsson, 2013) is a prospective study whose main objective is to assess how neonatal risk factors affect neurodevelopmental and neurodegenerative processes. It consisted initially of 1196 people born in 1971-1974 at the Helsinki metropolitan area maternity hospital (Kätilöopisto) who had at least one neonatal risk factor (e.g. birth weight < 2000g, need of external ventilation/ischaemia or asphyxia, Apgar < 7, neurological symptoms). Children were clinically examined at ages of 5, 9 and 16 and filled a questionnaire at 30. Possible ALS/FTLD diagnoses were derived from clinical records, medication reimbursement registry, neuropsychological examination and clinical examination by neurologist.

4.1.7. Blood donor cohort

The blood donor cohort consists of a collection of 400 healthy blood donors aged 18-65 years at the time of study participation, who were recruited in Northern Ostrobothnia (n=102), Kainuu (n=85), North Savo (n=61), Pirkanmaa (n=152) (Meinila, Finnila, & Majamaa, 2001; Niemi et al., 2003).

4.2. Genotyping and sequencing

DNA was extracted from venous blood samples (peripheral blood leukocytes) or in some individuals of the Vantaa85+ study from brain samples using standard methods. In the PLASTICITY study, DNA was extracted from saliva samples.

4.2.1. Vantaa85+ genotyping, sequencing and imputation

Genome-wide genotyping in 512 participants was done with the HumanCNV370 array (Illumina, CA, USA) according to the manufacturer's recommendations. BeadStudio v. 3.2 (Illumina) was used for reclustering with a no-call threshold of 0.15. Per-sample and per-variant quality control steps were: related individuals (identity by descent >0.185), individuals with divergent ancestry, individuals with discordant sex information, outlying heterozygosity rate (±2 SD) or elevated missing data (>3%) rate, and were excluded. Variants with missing per person rate >10%, missing data rate >5 %, minor allele frequency <1% or significantly different genotype call rates between cases and controls ($p < 0.00001$) were excluded. Variants not in Hardy-Weinberg equilibrium ($p < 0.00001$) were also excluded.

Whole-genome sequencing of 309 samples selected by DNA quality and quantity was done at Broad Institute. Samples were prepared with Illumina TruSeq DNA PCR Free library preparation kit and sequenced with the HiSeq X10 sequencing system to produce 150-base pair paired-end reads according to the manufacturer's protocol. We processed the raw sequence data using GATK (McKenna et al., 2010) according to the Broad Best Practices application program interface.

We performed whole-genome imputation using the HumanCNV370 array data and the population-specific SISu v3 imputation reference panel. First, in per-sample quality control, individuals with discordant sex information, excess heterozygosity (+-4SD) and high genotype missingness (>5%) were excluded. Then, in per-variant quality control, variants with high missingness (>2%), not in HWE (p<1e-6) and minor allele count < 3 were removed. The Vantaa85+ samples were merged with a larger dataset originating from a similar genotyping approach and pre-phased with Eagle 2.3.5 (https://data.broadinstitute.org/alkesgroup/Eagle/) with the default parameters, except the number of conditioning haplotypes was set to 20,000.

Two sets of imputations were done in Vantaa85. First, only AD risk loci were imputed. This was done using IMPUTE2. 1000 Genomes phase3 data (October 2014 release) supplied by IMPUTE2 were used as the reference panel.

Second, genome-wide imputation was done using the population-specific SISu v3 imputation reference panel with Beagle 4.1 (version 27Jan18.7e1, https://faculty.washington.edu/browning/beagle/b4_1.html) as described in the following protocol: dx.doi.org/10.17504/protocols.io.nmndc5e. High-coverage (25-30x) whole-genome sequence data was used to develop the SISu v3 reference panel (Pärn et al., manuscript in preparation). the variant callset was produced with GATK HaplotypeCaller algorithm by following the GATK best-practices for variant calling. Genotype-, sample- and variant-wise QC was applied in an iterative manner by using the Hail framework (https://github.com/hail-is/hail) v0.1 and the resulting high-quality WGS data for 3,775 individuals were phased with Eagle 2.3.5 as described above.

Post-imputation quality control consisted of discarding variants with an imputation info score < 0.3 and a minor allele frequency threshold of 0.01.

4.2.2. *APOE* genotyping

In all studies, *APOE* was genotyped with a PCR assay as previously described (Myllykangas et al., 1999). In brief, a PCR assay with HhAl restriction was used and the amplicons were visualized with agarose gel electrophoresis. Different amplicon lengths represent different genotypes. When possible, genotypes were double checked with genotyping or sequencing data.

4.2.3. *C9orf72* hexanucleotide repeat length assessment

The *C9orf72* hexanucleotide repeat length was determined with repeat-primed PCR and putative expansions were confirmed with over-the-repeat PCR as previously described (Renton et al., 2011). In brief, first repeat-primed PCR with fluorescent primers was performed and amplicons were then run on capillary electrophoresis. Then, putative expansions were verified with over-the-repeat PCR and compared to in-house control samples with known repeat lengths. In over-the-repeat PCR, the hexanucleotide repeat locus is amplified. Alleles with the expansion do not amplify since they are too long for the PCR. Samples possibly homozygous for intermediate repeat alleles were also verified with over-the-repeat PCR.

RESULTS AND DISCUSSION

4.1. C9orf72 repeat lengths in aged Finnish population and association with cognition

We characterized the repeat length of 3142 Finns from four cohorts: the Vantaa85, DEBATE, HBCS and HBS. The mean age was 78 years, age range was 58-101 and there were 49% women. The distribution of repeat lengths was largely the same between cohorts (Kruskal-Wallis H test, H (3) = 2.129, p = 0.55) except that all expansions came from the largest cohort (HBCS). All expansions were confirmed with over-the-repeat PCR.

The repeat length distribution showed peaks at 2(−3), 5, 8, and 10 repeats as previously described (Figure 11). The longer allele was in the range of 7–45 repeats in 1036/3142 (33%) individuals, 20–45 in 56/3142 (1.8%), 30–45 in 12/3142 (0.38%), and expansion (>45 repeats) in 6/3142 (0.19%).

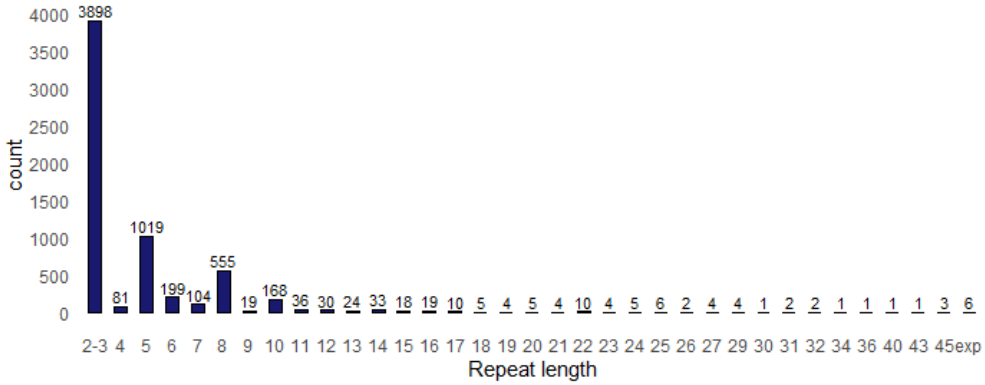

Figure 11. *C9orf72* repeat length distribution in aged Finnish individuals. Exp denotes an expansion.

Therefore, the allelic frequency of ≥20 repeats was 0.89% in Finland. This allelic frequency was found to be statistically significantly more common (all $p < 0.019$) than in four previous large (cohort studies of >1000 individuals or case-control studies with >1000 controls) studies where the allelic frequency was 0.38 – 0.52% (Table 4). The *C9orf72* hexanucleotide expansion is especially common in Finland (Majounie et al., 2012) and also the higher prevalence of ≥20 repeats, raises the thought that the longer repeat alleles could be prone to germline instability and becoming large expansions in offspring. A similar mechanism has been shown in Huntington's disease (Wheeler et al., 2007). This hypothesis could be studied

by assessing the repeat length of the parents of the *C9orf72* expansion positive patients. However, reliable results would require a large number of trios to be studied and since ALS is often diagnosed quite late in life, the patient's parents might no longer be alive. Potential mosaicism could also be a problem for interpreting the results if the repeat length was assessed only from blood-derived DNA.

Table 4. The frequency of long intermediate-length repeats in large control or general population.

Population	**Number of individuals**	**Number of ≥20 repeats (expansions excluded)**	**Allelic frequency of ≥20 repeats (%)**	***p*-value versus our sample**	**Expansions in controls (n)**	**Reference**
Finnish	3142	56	0.89		6	
British 1958 birth cohort	7577	64	0.42	0.000051	11	(Beck et al., 2013)
Irish	1234	10	0.41	0.019	0	(Fahey et al., 2014)
European/Asian/ North American/Australian	5886	61 (≥17 repeats)	0.52	0.0045	1	(Theuns et al., 2014)
North American	1444	11	0.38	0.0078	0	(Rutherford et al., 2012)

We also found that intermediate repeat lengths did not associate with cognitive impairment or AD (all $p > 0.05$). This lack of association held regardless of the threshold (7 or 20) for intermediate repeat length.

Moreover, contrary to the assumption of 30 repeats being pathogenic, we found 30-45 repeats in 0.38% (1 per 262) of individuals, and they did not have any apparent clustering of neurodegenerative or psychiatric diagnoses. The characterization of pathogenic threshold is important for several reasons. First, when counselling patients with suspected or confirmed ALS, it is important to known if their *C9orf72* repeat length is likely to be relevant to their disease or if other risk factors should be sought. This is also important in clinical trials when patients are pooled based on their primary mutation. Second, when studying the effects of different repeat lengths *in vitro* or *in vivo,* it is important to know what repeat lengths are relevant to study. Some intermediate-length repeat alleles may demonstrate an observable

biological change in comparison to wild type alleles but if those intermediate-length alleles do not associate with disease in humans, the effect is likely not to be clinically relevant.

Biological processes are seldom exact and the pathological threshold of repeat length can be a "gray zone" where disease penetrance is dependent on modulating cofactors. Statistical power is especially important to consider in negative result studies where a minute effect could have been left unseen. However, intermediate repeats are not a strong (relative risk >1.5) risk factor for AD or cognitive impairment.

The *C9orf72* hexanucleotide repeat expansion was discovered as late as in 2011 as a major cause for ALS and FTLD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Since neurodegenerative diseases share molecular mechanisms and several genes are known to be risk factors for multiple neurodegenerative diseases, it was natural that the association of *C9orf72* hexanucleotide repeat with various other neurodegenerative diseases was quickly tested. The results were mixed (Ng & Tan, 2017) as spurious positive associations are quite common as is a lack of sufficient power required for reliable negative results. Furthermore, as there is population-level variability in the prevalence of both expansion and intermediate alleles, population stratification needs to be considered. By studying a large number of Finnish individuals, we were able to control for population stratification and the higher than average prevalence of longer intermediate repeats allowed sufficient power. A limitation of our study was that we could not control for all important factors that affect cognitive impairment. One example is that the level of education was quite heterogeneous in our cohorts. Furthermore, there was variability in the assessment of cognition. Nevertheless, our data provide good evidence on the effects of *C9orf72* intermediate repeats on AD and cognitive impairment.

To date, there has not been any new data published that would contradict our results. The current view is that the *C9orf72* hexanucleotide expansion is at most a rare cause of AD (Kohli et al., 2013b; Majounie, Abramzon et al., 2012). Individuals clinically diagnosed with AD carrying the expansion may have in reality e.g. FTLD. AD can also resemble limbic-predominant age-related TAR-DNA-binding protein-43 (TDP-43) encephalopathy (LATE-NC) and its association with *C9orf72* has not yet been extensively studied. Nevertheless, the effects of intermediate repeats in AD seem to be quite negligible.

4.2. *C9orf72* intermediate length alleles and ALS

Since Publication I, new data suggesting the pathogenicity of *C9orf72* intermediate repeats were published, e.g. the meta-analysis of 24-30 repeats and ALS (Iacoangeli et al., 2019) and 17-30 repeats and corticobasal degeneration (Cali et al., 2019).

To study the effects of intermediate repeats on ALS, in addition to the 3142 older Finns we previously genotyped, we determined the *C9orf72* repeat length in 433 younger (mean age 42 years) Finns from the PLASTICITY study, 400 individuals from the blood donor cohort and 758 Finnish ALS patients from Southern Finland. Again, genotyping success rate was high (97-99%). Since the effect of an expansion most likely overshadows any effects of an intermediate repeat, we excluded expansion carriers from our primary analyses. After quality control steps, there were 525 ALS cases and 3950 controls for the main analyses.

As discussed earlier, there is no one definitive threshold for intermediate repeat length, so we chose several based on the level of previous evidence. The intermediate thresholds were 7-45, 17-45, 21-45, 24-45 and 24-30. Intermediate repeat length alleles did not associate with ALS (Fisher's test all $p \geq 0.15$) when the effect of the longer allele was considered (Table 5). We also tested if intermediate alleles had an impact on survival presenting as decline in the intermediate allele frequency in older age groups versus younger age groups. We distributed controls into three age groups (18-65, 66-84 and 85-105 years) and tested if there was a difference in the frequency of intermediate allele carriers. We did not observe a linear trend between age group and the frequency of intermediate-length allele carriers (Cochran-Armitage trend test, all $p \geq 0.33$) (Table 6) in any of the intermediate repeat length thresholds.

However, there was a difference in the proportion of individuals with two intermediate-length alleles between ALS cases and controls (p=0.005, OR 1.93, CI=1.19-3.02). The estimated effect increased with the repeat length of the longer allele (Table 7). We obtained similar results when testing the effect of the SNP rs3849942 whose allele A tags repeat lengths of ≥7. There were more individuals with ALS than controls with A/A genotype (p= 0.018, OR=1.89, 95%CI 1.20-2.99). This suggests that our intermediate-repeat assessment has been reliable even though samples with two intermediate-length alleles are difficult to genotype.

Taken together, our data suggest that one intermediate allele is not a risk factor for ALS in Finland, but carrying two intermediate-length alleles increases the risk of ALS. Our results are somewhat in contrast with some earlier studies, perhaps most notably the large meta-analysis of 5071 ALS cases and 3747 controls discussed earlier (Iacoangeli et al., 2019). However, this meta-analysis reported only the length of the longer allele. Other potential factors that may lead to differing results is the usage of different genotyping methods and population differences and population stratification. Our results agree with a Belgian study that reported that intermediate-length alleles increase the risk of ALS only in those with two intermediate-length alleles (OR 2.08, p=0.04) (Gijselinck et al., 2016). Moreover, in a Flanders-Belgian cohort, the A/A genotype of rs3849942 was more common in FTLD patients without the *C9orf72* repeat expansion than in controls (OR 1.75, p=0.04) (van der Zee et al., 2013).

The main limitations of this study were that achieving sufficient sample size requires pooling individuals from multiple cohorts. These cohorts had different demographic characteristics that can influence ALS risk and the rs3849942 genotyping platform varied.

In future, the role of homozygous intermediate genotypes in ALS is worth studying more with bigger sample sizes and slightly different methodology. Amplified fragment length polymorphism PCR with only two primers might have better sensitivity in detecting homozygous samples than RP-PCR but it has poorer expansion detection. Another interesting question is, if Finns have some enriched genetic variation that e.g. controls expression of intermediate repeats and therefore their effect on ALS development is smaller than in other populations. Targeted resequencing coupled with haplotype analysis could elucidate this question.

Table 5: Intermediate allele carrier frequencies in 525 ALS cases and 3950 controls (expansion carriers excluded).

Longer allele	**Controls n**	**ALS n**	**p**	**OR**	**95% CI**
7-45	1293 (33%)	185 (35%)	0.26	1.12	0.92-1.36
17-45	101 (2.6%)	19 (3.6%)	0.15	1.43	0.82-2.74
21-45	66 (1.7%)	12 (2.3%)	0.29	1.38	0.67-2.59
24-45	43 (1.1%)	6 (1.1%)	0.82	1.05	0.36-2.50
24-30	30 (0.76%)	3 (0.57%)	1	0.75	0.15-2.43

Table 6. Intermediate allele carrier frequencies in different control individual age groups.

	18-65 years	**66-84 years**	**85-105 years**	**p value**
7-45	273 (31%)	657 (33%)	363 (33%)	0.46
17-45	29 (3.3%)	44 (2.2%)	28 (2.5%)	0.33
21-45	18 (2.1%)	29 (1.5%)	19 (1.7%)	0.62
24-45	12 (1.4%)	17 (0.86%)	14 (1.3%)	0.92
24-30	10 (1.1%)	9 (0.46%)	11 (1.0%)	0.85
Size of age group	873	1973	1102	

Table 7. Individuals with two intermediate-length alleles in 525 ALS patients and 3 950 controls after exclusion of expansion carriers.

Shorter/longer allele	**Controls n (%)**	**ALS n (%)**	**p**	**OR**	**95% CI**
≥7/≥7	104 (2.6%)	26 (5.0%)	0.005	1.93	1.19-3.02
7-16/7-16	94 (2.4%)	19 (3.6%)	0.098	1.57	0.90- 2.62
≥7/17-45	10 (0.25%)	7 (1.3%)	0.0020	5.32	1.71-15.56
≥7/21-45	3 (0.076%)	6 (1.1%)	0.00016	15.19	3.23-94.21
≥7/24-45	1 (0.025%)	4 (0.76%)	0.00085	30.26	2.99-1479

4.3. Distribution and progression of LRP

In the neuropathologically examined Vantaa85+ subsample, LRP was common. It was observed in 124/304 (41%) of individuals. LRP severity in the different anatomic sites was scored semiquantitatively. This allowed for 113/124 of the samples to be classified according to the DLB consortium guidelines: 19 had brainstem type, 10 amygdala-predominant type, 41 limbic type, and 43 diffuse neocortical type.

In addition to the DLB consortium guideline types, LRP showed two progression patterns: caudo-rostral and amygdala based. All but one sample could be classified into these progression patterns. Caudo-rostral progression was more prevalent (83/123) but amygdala-based was also quite common, present in 40/123 (32%) of individuals with LRP.

There was clear overlap with the DLB consortium classification and LRP progression pattern: 95% of brainstem type had caudo-rostral progression and all amygdala-predominant type had the amygdala-base progression pattern. The overlap in limbic and diffuse neocortical types was more mixed.

Further studies showed that amygdala-based progression associated with AD pathology. The most severe Braak NFT stages (V–VI) were more commonly observed with the amygdala-based progression than with the caudo-rostral progression (Fisher's test, $p = 5.63e-8$) or with no LRP (Fisher's test, $p = 1.05e-7$). A moderate to frequent CERAD score was also more commonly observed with the amygdala-based progression than with the caudo-rostral progression (Fisher's test, $p=1.86e-5$) or with no LRP (Fisher's test, $p=6.91e-6$). There was no statistically significant difference (Fisher's test $p>0.05$) in Braak NFT stages V-VI or moderate to frequent CERAD score between the caudo-rostral pattern ($n = 83$) and individuals with no LRP ($n = 180$).

APOE ε4 genotype was more common in the amygdala-based progression pattern than in the caudo-rostral progression pattern (Fisher's test, $p = 0.001843$) or in individuals with no LRP (Fisher's test, $p = 4.61e-5$). There was no statistically significant difference in the *APOE ε4* genotype proportion between the caudo-rostral pattern and individuals with no LRP (Fisher's test, $p = 0.4457$).

In accordance with the previous results, significantly more people were demented in the amygdala-based progression pattern group than in the caudo-rostral group (Fisher's test, $p = 4.48e-4$) or in individuals with no LRP (Fisher's test, $p = 1.8e-5$). A summary of the results is presented in Table 7.

Table 7. Demographic, neuropathological and genetic differences between LRP classification schemes and their subgroups.

	No LRP	**(a) DLB Consortium classification n = 124**					**(b) LRP progression-based n = 123**	
	No LRP	**Non-classifiable**	**Brainstem**	**Amygdala-predominant**	**Limbic**	**Diffuse Neocortical**	**Caudo-rostral**	**Amygdala-based**
n	180	11	19	10	41	43	83	40
Women (%)	85	82	84	90	80	74	76	88
Mean age at death (years)	92.3	91.3	93.8	93.0	92.3	92.2	92.6	92.3
Age at death (n, %)								
85–89	45 (25)	5 (46)	4 (21)	1 (10)	14 (34)	13 (30)	26 (31)	11 (28)
90–94	94 (52)	3 (27)	9 (47)	6 (60)	16 (39)	18 (42)	33 (40)	18 (45)
≥ 95	41 (23)	3 (27)	6 (32)	3 (30)	11 (27)	12 (28)	24 (29)	11 (28)
Braak NFT stage (n, %)								
0-II	54 (30)	6 (55)	8 (42)	1 (10)	10 (24)	11 (26)	34 (41)	2 (5)
III–IV	92 (51)	3 (27)	8 (42)	5 (50)	20 (49)	14 (33)	36 (43)	13 (33)
V–VI	34 (19)	2 (18)	3 (16)	4 (40)	11 (27)	18 (42)	13 (16)	25 (63)
CERAD score (n, %)								
None	46 (26)	2 (18)	7 (37)	1 (10)	11 (27)	4 (9)	24 (29)	1 (3)
Sparse	24 (13)	1 (9)	3 (16)	0 (0)	3 (7)	2 (5)	9 (11)	0 (0)
Moderate–frequent	110 (61)	8 (73)	9 (47)	9 (90)	27 (66)	37 (86)	50 (60)	39 (98)
NIA-RI (n, %)								
No	32 (35)	3 (50)	7 (58)	0 (0)	7 (26)	4 (13)	21 (25)	0 (0)
Yes	59 (65)	3 (50)	5 (42)	7 (100)	20 (74)	26 (87)	24 (29)	36 (90)
Dementia status at death (n, %)								
No	74 (41)	5 (45)	10 (53)	1 (10)	15 (37)	3 (7)	31 (37)	3 (8)
Yes	106 (59)	6 (55)	9 (47)	9 (90)	26 (63)	40 (93)	52 (63)	37 (93)
***APOE ε4* (n, %)**								
No	126 (74)	7 (70)	15 (79)	5 (56)	19 (53)	22 (55)	54 (65)	13 (33)
Yes	44 (26)	3 (30)	4 (21)	4 (44)	17 (47)	18 (45)	24 (29)	22 (55)

DLB is the second most common primary neurodegenerative disease and our cohort study demonstrates again how common LRP is in very old populations. Despite being common, the etiology of DLB and LRP is still poorly understood as is its genetics. DLB has unclear overlap with AD and PDD, and so far the largest GWAS study on DLB found APOE, the most common risk factor for AD, to be the strongest association for DLB as well (Guerreiro et al., 2018). However, in our study, *APOE* did not universally associate with LRP as *APOE ε4* did not associate with the caudo-rostral progression pattern, but only with amygdala-based LRP. We did not study DLB per se but the differences in LRP progression point to a biologically distinct AD-associated LRP type.

Our findings raise the need to reanalyze DLB data with these two different subgroups in mind and provides an important piece of the puzzle in the relationship between *APOE*, AD pathology and α-synuclein pathology. One of the big questions has been whether APOE is an independent driver of α-synuclein pathology or whether its effects are mediated by AD pathology. Our data demonstrate that not all LRP is associated with *APOE ε4*, rather the association is seen in LRP when there is significant AD pathology. These findings suggest that *APOE ε4* does not independently drive α-synuclein pathology, rather it increases the likelihood of Aβ and tau pathology which in turn can increase α-synuclein pathology. Our results are in line with what has been previously reported for APOE and Parkinson's disease, another synucleinopathy like DLB: *APOE* was not associated with PD in a GWAS of tens of thousands of patients (Nalls et al., 2019). The caudo-rostral progression pattern of LRP is the one generally seen in PD and thus the lack of an association between *APOE* and PD is expected.

Limitations of the study include that the required in-depth neuropathological analysis limits sample size. When studying multiple subgroups, some subgroups are therefore quite small. The semiquantitative LRP scoring is also susceptible to some subjectivity that could be reduced by scanning pathological sections and using image analysis software coupled with machine-learning. Furthermore, since neuropathological follow-up studies demonstrate the actual progression of LRP in time in one individual are impossible, other methods are needed to validate progression patterns at an individual level. One possibility is MRI imaging with biomarkers for α-synuclein, tau and Aβ. Especially as magnetic flux densities of MRI machines increase, more detailed information can be gathered from living patients. In future, the genetics of DLB subtypes should be studied beyond *APOE* e.g. via GWAS. Also, by studying the AD and PD polygenic risk scores of the subtypes it could be possible to assess in more detail how the subtypes differ and if there is interaction between AD and PD risk factors.

4.4. Alzheimer's disease risk loci and neuropathological features

To study how AD genetic risk loci associate with the neuropathologic features of AD, we used the neuropathological data from Vantaa85+ coupled with genotyped and imputed SNV data. We compared individuals with moderate or frequent CERAD scores with individuals with no neuritic plaques (CERAD 0). We compared individuals with Braak stages 0-II with individuals with Braak high stage (stages IV-VI). We compared individuals with capillary Aβ to those without it. We studied CAA as a continuous variable. The percentage of blood vessels with CAA of all blood vessels visible in the tissue slide was used as the variable.

All candidate loci had variants either in the SNP array dataset or in the imputed dataset. We found an association ($p<0.05$) with most (24/29) AD risk loci with at least one of the AD pathological features. We could replicate the originally published index variants' association in 7 of 44 variants.

APOE ε4 was strongly associated with all the different neuropathological features (all $p< 2.01$ e-7 and all OR/$\beta> 6.49$). In addition to *APOE ε4, APP, MS4A* cluster, *NME8, SORL1*, the chromosome 9 locus and *SLC24A4* all associated with all neuropathological features.

The top association with CERAD was *MEF2C* (rs700588) (when adjusted for age, sex, and *APOE* ε4, $p = 0.0002122$, OR 2.67, 95% CI 1.59–4.49) Other loci that associated with CERAD were *ABCA7, ABCG1, APP, BIN1, FERMT2, GAB2, MSA4 CLUSTER, NME8, PICALM, PTK2B, SLC24A4*, *SORL1* and the chromosome 9 region.

The top association with Braak staging was found with *ABCG1* (rs532345, $p = 0.02671$, OR 0.5571, 95% CI 0.33–0.93 with APOE ε4 adjustment). Other associations were *ABCA7, APP, CASS4, CR1, GAB2, GALNT7, MEF2C, MS4A* cluster, *NME8, PTK2B, SLC24A4, SORL1*, the chromosome 9 region and *TRIP4*.

The top association with CAA was *CR1* (rs65087, $p = 0.004934$, β 2.52, 95% CI 0.78–4.26 without *APOE ε4* adjustment. The other associating loci were *ABCA7, ABCG1, APP, BIN1, CASS4, CD2AP, CLU, FERMT2, GAB2, GALNT7, HLA-DRB1, MSA4* cluster, *NME8, PICALM, SLC24A4, SORL1*, the chromosome 9 region, *TRIP4* and *ZCWPW1*.

The top association with capillary Aβ was *APP* (rs1783016, $p = 0.005933$, OR 2.01, 95% CI 1.22–3.30 with APOE ε4 adjustment). The other loci with association were *BIN1, CR1, FERMT2, GALNT7, HLA-DRB1, HLA-DRB5, MSA4 cluster, NME8, PICALM, PTK2B, SORL1,* the chromosome 9 region and *TREM2*.

The summary of association results are shown in Table 8.

Table 8. Summary of results. Results with (a) and without (b) *APOE ε4* carrier status as a covariate. Genes that associated with AD in the two new large AD GWAS meta-analyses (Jansen et al., 2019; Kunkle et al., 2019) are in bold.

	CapAβ[a]	CapAβ[b]	CAA[a]	CAA[b]	CERAD[a]	CERAD[b]	Braak[a]	Braak[b]	Variants at locus (n)
ABCA7			+	+	+		+	+	65
ABCG1			+	+	+	+	+	+	279
APP	+	+	+	+		+	+	+	733
BIN1	+	+	+	+	+	+			204
CASS4			+	+					52
CD2AP			+				+	+	363
CD33									10
CELF1									81
CLU			+						16
CR1		+	+	+			+	+	227
EPHA1									21
EXOC3L2									12
FERMT2	+	+	+	+	+	+			177
GAB2				+	+	+	+	+	237
GALNT7	+	+	+				+	+	199
HLADRB1	+	+	+	+					66
HLADRB5	+								10
INPP5D									26
MEF2C	+				+	+	+		295
***MS4A* cluster**	+	+	+		+	+	+	+	714
NME8	+	+	+	+	+	+	+	+	270
PICALM	+		+	+	+	+			400
PTK2B	+	+			+	+	+	+	459
SLC24A4		+	+	+	+	+	+	+	533
SORL1	+		+	+	+	+	+	+	242
Region in chr9	+	+	+	+	+	+	+	+	287
TREM2		+							2
TRIP4			+	+			+	+	49
ZCWPW1			+	+					32

Our results suggest that some AD loci play a role in the development of all AD neuropathological features while some with only one. This view is supported by new pathway analyses that show that some genes (e.g. *APOE*) are part of many of the AD molecular pathways whereas some are more specific to one pathway (Kunkle et al., 2019). We were among the first to study capillary Aβ and it was noteworthy that the associated loci of CAA and capAβ did not completely overlap. This suggests some differences in the underlying neuropathological mechanisms of these two neuropathologic changes.

This study has also limitations. Imputation was performed with a multinational reference panel. A population-specific imputation reference panel would most likely have allowed better imputation quality especially for rare variants. Another consideration is the need and methodology for multiple testing corrections. Since this was a replication study, we used p = 0.05 as the threshold for statistical significance. However, the larger and therefore the more variants a locus has, the more likely it is to have a variant to show spurious association. Newer bigger AD GWASes have also been published (Jansen et al., 2019; Kunkle et al., 2019) and not all loci we tested were replicated in them. These loci might be more population specific but, they may also be false positive observations in the previous studies. The newer AD GWASes also detected new loci that we did not study.

6. CONCLUSIONS

The aim of this study was to study the genetics of three neurodegenerative diseases: ALS, AD and DLB. Special emphasis was on the *C9orf72* hexanucleotide repeat and *APOE*.

Publication I:

Repeat lengths of over 20 seem to be more common in Finland than elsewhere, which raises the possibility that they function as premutations. The widely used pathogenic threshold of 30 repeats might be too low. There was no statistically significant association between intermediate repeats and AD/cognitive impairment.

Publication II:

After publication I, more evidence on the potential role of *C9orf72* intermediate repeats in the pathogenicity of ALS and CBD was reported. We found that carrying two intermediate-length alleles increases the risk of ALS whereas carrying just one intermediate-length allele does not.

Publication III:

We were able to confirm in a population-based study that there are two ways for LRP to progress: the caudo-rostral and amygdala-based patterns. There was a clear association of AD pathology with amygdala-based pattern but not with the caudo-rostral pattern. These findings confirm the previously hypothesized "Alzheimer associated LRP". It also raises the need to reanalyze DLB genetic data with these two progression patterns in mind.

Publication IV:

Different AD risk loci associate with different AD neuropathologic features. Some associate with all neuropathological features while others with only one. Moreover, we were able to show risk loci for capillary Aβ.

Overall, the results of this thesis elucidate the phenotypic effects of *C9orf72* intermediate-length alleles by showing that carrying one intermediate allele does not increase the risk of ALS or AD. However, carrying two intermediate-length alleles increases the risk of ALS. The results of this thesis also show that genes associated with AD show different association patterns with the neuropathological features of AD. Similarly, *APOE ε4,* the strongest association signal of DLB in multinational studies, associates only with one of the two progression patterns of LRP.

ACKNOWLEDGEMENTS

I want to sincerely thank my supervisors Professor Pentti Tienari and Adjunct Professor Liisa Myllykangas for giving me the opportunity to immerse myself in research and for all the help and guidance and for inspiring my interest in research. It has been truly inspiring to work under your supervision and you have given me the possibility to grow as a researcher. Luckily, even after my doctoral defence I have the opportunity to continue to work with you and keep learning from you.

My sincere gratitude goes also to my steering committee, to Professor Anna-Elina Lehesjoki and Professor Hannes Lohi for their support. I want to also thank the reviewers of this thesis, Adjunct Professor Annakaisa Haapasalo and Adjunct Professor Mikko Kärppä for their insightful and encouraging comments.

From Pentti's group, the guidance of Lilja Jansson in the laboratory and the help of Miko Valori in bioinformatics and statistics have been crucial for this thesis. I would also like to thank Anna Kiviharju for her guidance and knowledge on the assessment of *C9orf72* repeats that she was willing to share even after graduating and transferring workplace.

A huge thank you to all my other co-authors, especially to Anna Raunio, Mia Kero and Mira Mäkelä whose projects allowed me to get insight into the world of neuropathology.

I am of course grateful to my whole family and to my friends who have supported me and believed in me and listened to all those little (or longer) talks about my research even though they might not have been that interested in the topic.

This work was carried out in Helsinki University, in Molecular Neurology and subsequently in Translational immunology research programs. The department of pathology, HUSLAB was also an important part of this study. I'm also very grateful for the opportunity of visiting the National Institutes of Health where the guidance and support of Dr. Bryan Traynor and especially Dr. Sonja Sholz and her research group members was important, and you all made me feel welcome.

My work was financially supported by The Finnish Cultural Foundation, Maire Taponen Foundation, The Finnish Medical Foundation, Sigrid Jusélius Foundation, the Paulo foundation and the Finnish Brain Foundation sr. The Helsinki University MD-PhD program also funded my work and gave the unique possibility of getting to know and working in different research groups.

REFERENCES

References

1000 Genomes Project Consortium, Auton, A., Brooks, L. D., Durbin, R. M., Garrison, E. P., Kang, H. M., . . . Abecasis, G. R. (2015). A global reference for human genetic variation. *Nature, 526*(7571), 68-74. doi:10.1038/nature15393 [doi]

Al-Chalabi, A., Calvo, A., Chio, A., Colville, S., Ellis, C. M., Hardiman, O., . . . Pearce, N. (2014). Analysis of amyotrophic lateral sclerosis as a multistep process: A population-based modelling study. *The Lancet.Neurology, 13*(11), 1108-1113. doi:S1474-4422(14)70219-4 [pii]

Al-Chalabi, A., Hardiman, O., Kiernan, M. C., Chio, A., Rix-Brooks, B., & van den Berg, L H. (2016). Amyotrophic lateral sclerosis: Moving towards a new classification system. *The Lancet.Neurology, 15*(11), 1182-1194. doi:10.1016/S1474-4422(16)30199-5 [doi]

Al-Chalabi, A., Jones, A., Troakes, C., King, A., Al-Sarraj, S., & van den Berg, L H. (2012). The genetics and neuropathology of amyotrophic lateral sclerosis. *Acta Neuropathologica, 124*(3), 339-352. doi:10.1007/s00401-012-1022-4 [doi]

Ali, K., Middleton, M., Pure, E., & Rader, D. J. (2005). Apolipoprotein E suppresses the type I inflammatory response in vivo. *Circulation Research, 97*(9), 922-927. doi:01.RES.0000187467.67684.43 [pii]

Allen, M., Kachadoorian, M., Quicksall, Z., Zou, F., Chai, H. S., Younkin, C., . . . Ertekin-Taner, N. (2014). Association of MAPT haplotypes with alzheimer's disease risk and MAPT brain gene expression levels. *Alzheimer's Research & Therapy, 6*(4), 39. doi:10.1186/alzrt268 [doi]

Amouyel, P., Brousseau, T., Fruchart, J. C., & Dallongeville, J. (1993). Apolipoprotein E-epsilon 4 allele and alzheimer's disease. *Lancet (London, England), 342*(8882), 1309.

Andersen, P. M. (2006). Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Current Neurology and Neuroscience Reports, 6*(1), 37-46. doi:10.1007/s11910-996-0008-9 [doi]

Anderson, J. P., Walker, D. E., Goldstein, J. M., de Laat, R., Banducci, K., Caccavello, R. J., . . . Chilcote, T. J. (2006). Phosphorylation of ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic lewy body disease. *The Journal of Biological Chemistry, 281*(40), 29739-29752. doi:M600933200 [pii]

Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., . . . Oda, T. (2006). TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochemical and Biophysical Research Communications, 351*(3), 602-611. doi:S0006-291X(06)02318-7 [pii]

Armon, C. (2009). Smoking may be considered an established risk factor for sporadic ALS. *Neurology, 73*(20), 1693-1698. doi:10.1212/WNL.0b013e3181c1df48 [doi]

Arvanitakis, Z., Leurgans, S. E., Wang, Z., Wilson, R. S., Bennett, D. A., & Schneider, J. A. (2011). Cerebral amyloid angiopathy pathology and cognitive domains in older persons. *Annals of Neurology, 69*(2), 320-327. doi:10.1002/ana.22112 [doi]

Attems, J., Yamaguchi, H., Saido, T. C., & Thal, D. R. (2010). Capillary CAA and perivascular abeta-deposition: Two distinct features of alzheimer's disease pathology. *Journal of the Neurological Sciences, 299*(1-2), 155-162. doi:10.1016/j.jns.2010.08.030 [doi]

Attems, J., Jellinger, K., Thal, D. R., & Van Nostrand, W. (2011). Review: Sporadic cerebral amyloid angiopathy. *Neuropathology and Applied Neurobiology, 37*(1), 75-93. doi:10.1111/j.1365-2990.2010.01137.x [doi]

Ayers, J. I., Fromholt, S. E., O'Neal, V. M., Diamond, J. H., & Borchelt, D. R. (2016). Prion-like propagation of mutant SOD1 misfolding and motor neuron disease spread along neuroanatomical pathways. *Acta Neuropathologica, 131*(1), 103-114. doi:10.1007/s00401-015-1514-0 [doi]

Balendra, R., & Isaacs, A. M. (2018). C9orf72-mediated ALS and FTD: Multiple pathways to disease. *Nature Reviews.Neurology, 14*(9), 544-558. doi:10.1038/s41582-018-0047-2 [doi]

Bandres-Ciga, S., Noyce, A. J., Hemani, G., Nicolas, A., Calvo, A., Mora, G., . . . Traynor, B. J. (2019). Shared polygenic risk and causal inferences in amyotrophic lateral sclerosis. *Annals of Neurology, 85*(4), 470-481. doi:10.1002/ana.25431 [doi]

Barker, W. W., Luis, C. A., Kashuba, A., Luis, M., Harwood, D. G., Loewenstein, D., . . . Duara, R. (2002). Relative frequencies of alzheimer disease, lewy body, vascular and

frontotemporal dementia, and hippocampal sclerosis in the state of florida brain bank. *Alzheimer Disease and Associated Disorders, 16*(4), 203-212. doi:10.1097/00002093-200210000-00001 [doi]

Barmada, S. J., Skibinski, G., Korb, E., Rao, E. J., Wu, J. Y., & Finkbeiner, S. (2010). Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral sclerosis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 30*(2), 639-649. doi:10.1523/JNEUROSCI.4988-09.2010 [doi]

Bartus, R. T., Dean, R. L., Beer, B., & Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science (New York, N.Y.), 217*(4558), 408-414. doi:10.1126/science.7046051 [doi]

Bassil, F., Brown, H. J., Pattabhiraman, S., Iwasyk, J. E., Maghames, C. M., Meymand, E. S., . . . Lee, V. M. (2019). Amyloid-beta (abeta) plaques promote seeding and spreading of alpha-synuclein and tau in a mouse model of lewy body disorders with abeta pathology. *Neuron,* doi:S0896-6273(19)30883-9 [pii]

Beecham, G. W., Hamilton, K., Naj, A. C., Martin, E. R., Huentelman, M., Myers, A. J., . . . Montine, T. J. (2014). Genome-wide association meta-analysis of neuropathologic features of alzheimer's disease and related dementias. *PLoS Genetics, 10*(9), e1004606. doi:10.1371/journal.pgen.1004606 [doi]

Bensimon, G., Lacomblez, L., & Meininger, V. (1994). A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/riluzole study group. *The New England Journal of Medicine, 330*(9), 585-591. doi:10.1056/NEJM199403033300901 [doi]

Biernat, J., Mandelkow, E. M., Schroter, C., Lichtenberg-Kraag, B., Steiner, B., Berling, B., . . . Mandelkow, E. (1992). The switch of tau protein to an alzheimer-like state includes the phosphorylation of two serine-proline motifs upstream of the microtubule binding region. *The EMBO Journal, 11*(4), 1593-1597.

Blokhuis, A. M., Groen, E. J., Koppers, M., van den Berg, L H, & Pasterkamp, R. J. (2013). Protein aggregation in amyotrophic lateral sclerosis. *Acta Neuropathologica, 125*(6), 777-794. doi:10.1007/s00401-013-1125-6 [doi]

Boeve, B. F., Silber, M. H., Ferman, T. J., Lin, S. C., Benarroch, E. E., Schmeichel, A. M., . . . Dickson, D. W. (2013). Clinicopathologic correlations in 172 cases of rapid eye movement sleep behavior disorder with or without a coexisting neurologic disorder. *Sleep Medicine, 14*(8), 754-762. doi:10.1016/j.sleep.2012.10.015 [doi]

Boivin, M., Pfister, V., Gaucherot, A., Ruffenach, F., Negroni, L., Sellier, C., & Charlet-Berguerand, N. (2020). Reduced autophagy upon C9ORF72 loss synergizes with dipeptide repeat protein toxicity in G4C2 repeat expansion disorders. *The EMBO Journal, 39*(4), e100574. doi:10.15252/embj.2018100574 [doi]

Boot, B. P., Orr, C. F., Ahlskog, J. E., Ferman, T. J., Roberts, R., Pankratz, V. S., . . . Boeve, B. F. (2013). Risk factors for dementia with lewy bodies: A case-control study. *Neurology, 81*(9), 833-840. doi:10.1212/WNL.0b013e3182a2cbd1 [doi]

Bourinaris, T., & Houlden, H. (2018). C9orf72 and its relevance in parkinsonism and movement disorders: A comprehensive review of the literature. *Movement Disorders Clinical Practice, 5*(6), 575-585. doi:10.1002/mdc3.12677 [doi]

Braak, H., & Braak, E. (1995). Staging of alzheimer's disease-related neurofibrillary changes. *Neurobiology of Aging, 16*(3), 271-84. doi:0197458095000216 [pii]

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., & Del Tredici, K. (2006). Staging of alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathologica, 112*(4), 389-404. doi:10.1007/s00401-006-0127-z [doi]

Braak, H., Del Tredici, K., Rub, U., de Vos, R. A., Jansen Steur, E. N., & Braak, E. (2003). Staging of brain pathology related to sporadic parkinson's disease. *Neurobiology of Aging, 24*(2), 197-211. doi:S0197458002000659 [pii]

Burberry, A., Suzuki, N., Wang, J. Y., Moccia, R., Mordes, D. A., Stewart, M. H., . . . Eggan, K. (2016). Loss-of-function mutations in the C9ORF72 mouse ortholog cause fatal autoimmune disease. *Science Translational Medicine, 8*(347), 347ra93. doi:10.1126/scitranslmed.aaf6038 [doi]

Byrne, S., Walsh, C., Lynch, C., Bede, P., Elamin, M., Kenna, K., . . . Hardiman, O. (2011). Rate of familial amyotrophic lateral sclerosis: A systematic review and meta-analysis. *Journal of Neurology, Neurosurgery, and Psychiatry, 82*(6), 623-627. doi:10.1136/jnnp.2010.224501 [doi]

Cacace, R., Sleegers, K., & Van Broeckhoven, C. (2016). Molecular genetics of early-onset alzheimer's disease revisited. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association, 12*(6), 733-748. doi:10.1016/j.jalz.2016.01.012 [doi]

Cali, C. P., Patino, M., Tai, Y. K., Ho, W. Y., McLean, C. A., Morris, C. M., . . . Lee, E. B. (2019). C9orf72 intermediate repeats are associated with corticobasal degeneration, increased C9orf72 expression and disruption of autophagy. *Acta Neuropathologica, 138*(5), 795-811. doi:10.1007/s00401-019-02045-5 [doi]

Cavalli, G., & Heard, E. (2019). Advances in epigenetics link genetics to the environment and disease. *Nature, 571*(7766), 489-499. doi:10.1038/s41586-019-1411-0 [doi]

Chang, D., Nalls, M. A., Hallgrimsdottir, I. B., Hunkapiller, J., van der Brug, M., Cai, F., . . . Graham, R. R. (2017). A meta-analysis of genome-wide association studies identifies 17 new parkinson's disease risk loci. *Nature Genetics, 49*(10), 1511-1516. doi:10.1038/ng.3955 [doi]

Chartier-Harlin, M. C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L., . . . Hardy, J. (1991). Early-onset alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature, 353*(6347), 844-846. doi:10.1038/353844a0 [doi]

Chen, Y., Lin, Z., Chen, X., Cao, B., Wei, Q., Ou, R., . . . Shang, H. F. (2016). Large C9orf72 repeat expansions are seen in chinese patients with sporadic amyotrophic lateral sclerosis. *Neurobiology of Aging, 38*, 217.e15-217.e22. doi:S0197-4580(15)00589-8 [pii]

Chia, R., Chio, A., & Traynor, B. J. (2018). Novel genes associated with amyotrophic lateral sclerosis: Diagnostic and clinical implications. *The Lancet.Neurology, 17*(1), 94-102. doi:S1474-4422(17)30401-5 [pii]

Chio, A., Logroscino, G., Traynor, B. J., Collins, J., Simeone, J. C., Goldstein, L. A., & White, L. A. (2013). Global epidemiology of amyotrophic lateral sclerosis: A systematic review of the published literature. *Neuroepidemiology, 41*(2), 118-130. doi:10.1159/000351153 [doi]

Clarimon, J., Molina-Porcel, L., Gomez-Isla, T., Blesa, R., Guardia-Laguarta, C., Gonzalez-Neira, A., . . . Lleo, A. (2009). Early-onset familial lewy body dementia with extensive tauopathy: A clinical, genetic, and neuropathological study. *Journal of Neuropathology and Experimental Neurology, 68*(1), 73-82. doi:10.1097/NEN.0b013e3181927577 [doi]

Collins, R. L., Brand, H., Karczewski, K. J., Zhao, X., Alfoldi, J., Francioli, L. C., . . . Talkowski, M. E. (2020). A structural variation reference for medical and population genetics. *Nature, 581*(7809), 444-451. doi:10.1038/s41586-020-2287-8 [doi]

Consensus recommendations for the postmortem diagnosis of alzheimer's disease. the national institute on aging, and reagan institute working group on diagnostic criteria for the neuropathological assessment of alzheimer's disease. (1997). *Neurobiology of Aging, 18*(4 Suppl), 1. doi:S0197-4580(97)00057-2 [pii]

Crockford, C., Newton, J., Lonergan, K., Chiwera, T., Booth, T., Chandran, S., . . . Abrahams, S. (2018). ALS-specific cognitive and behavior changes associated with advancing disease

stage in ALS. *Neurology, 91*(15), e1370-e1380. doi:10.1212/WNL.0000000000006317 [doi]

Davis, A. A., Inman, C. E., Wargel, Z. M., Dube, U., Freeberg, B. M., Galluppi, A., . . . Holtzman, D. M. (2020). APOE genotype regulates pathology and disease progression in synucleinopathy. *Science Translational Medicine, 12*(529), 10.1126/scitranslmed.aay3069. doi:eaay3069 [pii]

Deelen, J., Evans, D. S., Arking, D. E., Tesi, N., Nygaard, M., Liu, X., . . . Murabito, J. M. (2019). A meta-analysis of genome-wide association studies identifies multiple longevity genes. *Nature Communications, 10*(1), 3669-2. doi:10.1038/s41467-019-11558-2 [doi]

DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Rutherford, N. J., . . . Rademakers, R. (2011). Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron, 72*(2), 245-256. doi:10.1016/j.neuron.2011.09.011 [doi]

Desplats, P., Lee, H. J., Bae, E. J., Patrick, C., Rockenstein, E., Crews, L., . . . Lee, S. J. (2009). Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proceedings of the National Academy of Sciences of the United States of America, 106*(31), 13010-13015. doi:10.1073/pnas.0903691106 [doi]

Dickson, D. W., Heckman, M. G., Murray, M. E., Soto, A. I., Walton, R. L., Diehl, N. N., . . . Ross, O. A. (2018). APOE epsilon4 is associated with severity of lewy body pathology independent of alzheimer pathology. *Neurology, 91*(12), e1182-e1195. doi:10.1212/WNL.0000000000006212 [doi]

Dickson, D. W., Uchikado, H., Fujishiro, H., & Tsuboi, Y. (2010). Evidence in favor of braak staging of parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society, 25 Suppl 1*, 78. doi:10.1002/mds.22637 [doi]

Dong, X. X., Wang, Y., & Qin, Z. H. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica, 30*(4), 379-387. doi:10.1038/aps.2009.24 [doi]

Donnelly, C. J., Zhang, P. W., Pham, J. T., Haeusler, A. R., Mistry, N. A., Vidensky, S., . . . Rothstein, J. D. (2013). RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. *Neuron, 80*(2), 415-428. doi:10.1016/j.neuron.2013.10.015 [doi]

Duggan, M., Torkzaban, B., Ahooyi, T. M., Khalili, K., & Gordon, J. (2019). Age-related neurodegenerative diseases. *Journal of Cellular Physiology,* doi:10.1002/jcp.29248 [doi]

Eisenberg, D. T., Kuzawa, C. W., & Hayes, M. G. (2010). Worldwide allele frequencies of the human apolipoprotein E gene: Climate, local adaptations, and evolutionary history. *American Journal of Physical Anthropology, 143*(1), 100-111. doi:10.1002/ajpa.21298 [doi]

Elobeid, A., Libard, S., Leino, M., Popova, S. N., & Alafuzoff, I. (2016). Altered proteins in the aging brain. *Journal of Neuropathology and Experimental Neurology, 75*(4), 316-325. doi:10.1093/jnen/nlw002 [doi]

Ferman, T. J., Boeve, B. F., Smith, G. E., Lin, S. C., Silber, M. H., Pedraza, O., . . . Dickson, D. W. (2011). Inclusion of RBD improves the diagnostic classification of dementia with lewy bodies. *Neurology, 77*(9), 875-882. doi:10.1212/WNL.0b013e31822c9148 [doi]

Feussner, G., Wey, S., Bommer, J., Deppermann, D., Grutzmacher, P., & Ziegler, R. (1992). Apolipoprotein E phenotypes and hyperlipidemia in patients under maintenance hemodialysis. *Human Genetics, 88*(3), 307-312. doi:10.1007/BF00197265 [doi]

Finkel, R. S., Mercuri, E., Darras, B. T., Connolly, A. M., Kuntz, N. L., Kirschner, J., . . . ENDEAR Study Group. (2017). Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *The New England Journal of Medicine, 377*(18), 1723-1732. doi:10.1056/NEJMoa1702752 [doi]

Forsberg, K., Graffmo, K., Pakkenberg, B., Weber, M., Nielsen, M., Marklund, S., . . . Andersen, P. M. (2019). Misfolded SOD1 inclusions in patients with mutations in C9orf72 and other ALS/FTD-associated genes. *Journal of Neurology, Neurosurgery, and Psychiatry, 90*(8), 861-869. doi:10.1136/jnnp-2018-319386 [doi]

Gallagher, M. D., & Chen-Plotkin, A. S. (2018). The post-GWAS era: From association to function. *American Journal of Human Genetics, 102*(5), 717-730. doi:S0002-9297(18)30134-4 [pii]

Galvin, J. E., Lee, S. L., Perry, A., Havlioglu, N., McKeel, D. W., & Morris, J. C. (2002). Familial dementia with lewy bodies: Clinicopathologic analysis of two kindreds. *Neurology, 59*(7), 1079-1082. doi:10.1212/wnl.59.7.1079 [doi]

Gamba, G. (2001). Initial sequence and analysis of the human genome. [La secuencia y analisis inicial del genoma humano] *Revista De Investigacion Clinica; Organo Del Hospital De Enfermedades De La Nutricion, 53*(4), 294-297.

Gatz, M., Reynolds, C. A., Fratiglioni, L., Johansson, B., Mortimer, J. A., Berg, S., . . . Pedersen, N. L. (2006). Role of genes and environments for explaining alzheimer disease. *Archives of General Psychiatry, 63*(2), 168-174. doi:63/2/168 [pii]

Ghiselli, G., Schaefer, E. J., Gascon, P., & Breser, H. B. (1981). Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science (New York, N.Y.), 214*(4526), 1239-1241. doi:10.1126/science.6795720 [doi]

Gijselinck, I., Van Mossevelde, S., van der Zee, J., Sieben, A., Engelborghs, S., De Bleecker, J., . . . Van Broeckhoven, C. (2016). The C9orf72 repeat size correlates with onset age of disease, DNA methylation and transcriptional downregulation of the promoter. *Molecular Psychiatry, 21*(8), 1112-1124. doi:10.1038/mp.2015.159 [doi]

Glenner, G. G., & Wong, C. W. (1984). Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications, 120*(3), 885-890. doi:S0006-291X(84)80190-4 [pii]

Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., . . . James, L. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial alzheimer's disease. *Nature, 349*(6311), 704-706. doi:10.1038/349704a0 [doi]

Gold, M., Hurwitz, J., & Anders, M. (1963). The enzymatic methylation of RNA and DNA. *Biochemical and Biophysical Research Communications, 11*, 107-114. doi:10.1016/0006-291x(63)90075-5 [doi]

Grassi, D., Howard, S., Zhou, M., Diaz-Perez, N., Urban, N. T., Guerrero-Given, D., . . . Lasmezas, C. I. (2018). Identification of a highly neurotoxic alpha-synuclein species inducing mitochondrial damage and mitophagy in parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 115*(11), E2634-E2643. doi:10.1073/pnas.1713849115 [doi]

Greten-Harrison, B., Polydoro, M., Morimoto-Tomita, M., Diao, L., Williams, A. M., Nie, E. H., . . . Chandra, S. S. (2010). Alphabetagamma-synuclein triple knockout mice reveal age-dependent neuronal dysfunction. *Proceedings of the National Academy of Sciences of the United States of America, 107*(45), 19573-19578. doi:10.1073/pnas.1005005107 [doi]

Guerreiro, R., Escott-Price, V., Hernandez, D. G., Kun-Rodrigues, C., Ross, O. A., Orme, T., . . . Bras, J. (2019). Heritability and genetic variance of dementia with lewy bodies. *Neurobiology of Disease, 127*, 492-501. doi:S0969-9961(19)30095-6 [pii]

Guerreiro, R., Ross, O. A., Kun-Rodrigues, C., Hernandez, D. G., Orme, T., Eicher, J. D., . . . Bras, J. (2018). Investigating the genetic architecture of dementia with lewy bodies: A two-stage genome-wide association study. *The Lancet.Neurology, 17*(1), 64-74. doi:S1474-4422(17)30400-3 [pii]

Guyant-Marechal, I., Berger, E., erriere, A., Rovelet-Lecrux, A., Viennet, G., Frebourg, T., . . . Hannequin, D. (2008). Intrafamilial diversity of phenotype associated with app duplication. *Neurology, 71*(23), 1925-1926. doi:10.1212/01.wnl.0000339400.64213.56 [doi]

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: Lessons from the alzheimer's amyloid beta-peptide. *Nature Reviews.Molecular Cell Biology, 8*(2), 101-112. doi:nrm2101 [pii]

Haidet-Phillips, A. M., Hester, M. E., Miranda, C. J., Meyer, K., Braun, L., Frakes, A., . . . Kaspar, B. K. (2011). Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nature Biotechnology, 29*(9), 824-828. doi:10.1038/nbt.1957 [doi]

Hamilton, R. L. (2000). Lewy bodies in alzheimer's disease: A neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. *Brain Pathology (Zurich, Switzerland), 10*(3), 378-384. doi:10.1111/j.1750-3639.2000.tb00269.x [doi]

Harding, A. J., Broe, G. A., & Halliday, G. M. (2002). Visual hallucinations in lewy body disease relate to lewy bodies in the temporal lobe. *Brain : A Journal of Neurology, 125*(Pt 2), 391-403. doi:10.1093/brain/awf033 [doi]

Harper, L., Fumagalli, G. G., Barkhof, F., Scheltens, P., O'Brien, J. T., Bouwman, F., . . . Schott, J. M. (2016). MRI visual rating scales in the diagnosis of dementia: Evaluation in 184 post-mortem confirmed cases. *Brain : A Journal of Neurology, 139*(Pt 4), 1211-1225. doi:10.1093/brain/aww005 [doi]

Hecht, M. J., Fellner, F., Fellner, C., Hilz, M. J., Heuss, D., & Neundorfer, B. (2001). MRI-FLAIR images of the head show corticospinal tract alterations in ALS patients more frequently than T2-, T1- and proton-density-weighted images. *Journal of the Neurological Sciences, 186*(1-2), 37-44. doi:S0022510X01005032 [pii]

Hirano, A., & Zimmerman, H. M. (1962). Alzheimer's neurofibrillary changes. A topographic study. *Archives of Neurology, 7*, 227-242. doi:10.1001/archneur.1962.04210030065009 [doi]

Hokkanen, L., Launes, J., & Michelsson, K. (2013). The perinatal adverse events and special trends in cognitive trajectory (PLASTICITY) - pre-protocol for a prospective longitudinal follow-up cohort study. *F1000Research, 2*, 50-50.v1. eCollection 2013. doi:10.12688/f1000research.2-50.v1 [doi]

Huebbe, P., & Rimbach, G. (2017). Evolution of human apolipoprotein E (APOE) isoforms: Gene structure, protein function and interaction with dietary factors. *Ageing Research Reviews, 37*, 146-161. doi:S1568-1637(17)30080-6 [pii]

Iacoangeli, A., Al Khleifat, A., Jones, A. R., Sproviero, W., Shatunov, A., Opie-Martin, S., . . . Al-Chalabi, A. (2019). C9orf72 intermediate expansions of 24-30 repeats are associated with ALS. *Acta Neuropathologica Communications, 7*(1), 115-4. doi:10.1186/s40478-019-0724-4 [doi]

Iljina, M., Garcia, G. A., Horrocks, M. H., Tosatto, L., Choi, M. L., Ganzinger, K. A., . . . Klenerman, D. (2016). Kinetic model of the aggregation of alpha-synuclein provides

insights into prion-like spreading. *Proceedings of the National Academy of Sciences of the United States of America, 113*(9), 1206. doi:10.1073/pnas.1524128113 [doi]

Ince, P. G., McArthur, F. K., Bjertness, E., Torvik, A., Candy, J. M., & Edwardson, J. A. (1995). Neuropathological diagnoses in elderly patients in oslo: Alzheimer's disease, lewy body disease, vascular lesions. *Dementia (Basel, Switzerland), 6*(3), 162-168. doi:10.1159/000106940 [doi]

Ingre, C., Roos, P. M., Piehl, F., Kamel, F., & Fang, F. (2015). Risk factors for amyotrophic lateral sclerosis. *Clinical Epidemiology, 7*, 181-193. doi:10.2147/CLEP.S37505 [doi]

International Human Genome Sequencing Consortium. (2004). Finishing the euchromatic sequence of the human genome. *Nature, 431*(7011), 931-945. doi:nature03001 [pii]

Irwin, D. J., Xie, S. X., Coughlin, D., Nevler, N., Akhtar, R. S., McMillan, C. T., . . . Trojanowski, J. Q. (2018). CSF tau and beta-amyloid predict cerebral synucleinopathy in autopsied lewy body disorders. *Neurology, 90*(12), e1038-e1046. doi:10.1212/WNL.0000000000005166 [doi]

Jansen, I. E., Savage, J. E., Watanabe, K., Bryois, J., Williams, D. M., Steinberg, S., . . . Posthuma, D. (2019). Genome-wide meta-analysis identifies new loci and functional pathways influencing alzheimer's disease risk. *Nature Genetics, 51*(3), 404-413. doi:10.1038/s41588-018-0311-9 [doi]

Jellinger, K. A. (2003). Neuropathological spectrum of synucleinopathies. *Movement Disorders : Official Journal of the Movement Disorder Society, 18 Suppl 6*, 2. doi:10.1002/mds.10557 [doi]

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P. V., Snaedal, J., . . . Stefansson, K. (2013). Variant of TREM2 associated with the risk of alzheimer's disease. *The New England Journal of Medicine, 368*(2), 107-116. doi:10.1056/NEJMoa1211103 [doi]

Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., . . . Muller-Hill, B. (1987). The precursor of alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature, 325*(6106), 733-736. doi:10.1038/325733a0 [doi]

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alfoldi, J., Wang, Q., . . . MacArthur, D. G. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature, 581*(7809), 434-443. doi:10.1038/s41586-020-2308-7 [doi]

Katsnelson, A., De Strooper, B., & Zoghbi, H. Y. (2016). Neurodegeneration: From cellular concepts to clinical applications. *Science Translational Medicine, 8*(364), 364ps18. doi:8/364/364ps18 [pii]

Keage, H. A., Carare, R. O., Friedland, R. P., Ince, P. G., Love, S., Nicoll, J. A., . . . Brayne, C. (2009). Population studies of sporadic cerebral amyloid angiopathy and dementia: A systematic review. *BMC Neurology, 9*, 3-3. doi:10.1186/1471-2377-9-3 [doi]

Kerminen, S., Havulinna, A. S., Hellenthal, G., Martin, A. R., Sarin, A. P., Perola, M., . . . Pirinen, M. (2017). Fine-scale genetic structure in finland. *G3 (Bethesda, Md.), 7*(10), 3459-3468. doi:10.1534/g3.117.300217 [doi]

Kirkwood, T. B. (2005). Understanding the odd science of aging. *Cell, 120*(4), 437-447. doi:S0092-8674(05)00101-7 [pii]

Klim, J. R., Vance, C., & Scotter, E. L. (2019). Antisense oligonucleotide therapies for amyotrophic lateral sclerosis: Existing and emerging targets. *The International Journal of Biochemistry & Cell Biology, 110*, 149-153. doi:S1357-2725(19)30062-7 [pii]

Kohli, M. A., John-Williams, K., Rajbhandary, R., Naj, A., Whitehead, P., Hamilton, K., . . . Zuchner, S. (2013a). Repeat expansions in the C9ORF72 gene contribute to alzheimer's disease in caucasians. *Neurobiology of Aging, 34*(5), 1519.e5-1519.12. doi:10.1016/j.neurobiolaging.2012.10.003 [doi]

Kohli, M. A., John-Williams, K., Rajbhandary, R., Naj, A., Whitehead, P., Hamilton, K., . . . Zuchner, S. (2013b). Repeat expansions in the C9ORF72 gene contribute to alzheimer's disease in caucasians. *Neurobiology of Aging, 34*(5), 1519.e5-1519.12. doi:10.1016/j.neurobiolaging.2012.10.003 [doi]

Kok, E., Haikonen, S., Luoto, T., Huhtala, H., Goebeler, S., Haapasalo, H., & Karhunen, P. J. (2009). Apolipoprotein E-dependent accumulation of alzheimer disease-related lesions begins in middle age. *Annals of Neurology, 65*(6), 650-657. doi:10.1002/ana.21696 [doi]

Komatsu, J., Samuraki, M., Nakajima, K., Arai, H., Arai, H., Arai, T., . . . Yamada, M. (2018). (123)I-MIBG myocardial scintigraphy for the diagnosis of DLB: A multicentre 3-year follow-up study. *Journal of Neurology, Neurosurgery, and Psychiatry, 89*(11), 1167-1173. doi:10.1136/jnnp-2017-317398 [doi]

Kornhuber, J., Weller, M., Schoppmeyer, K., & Riederer, P. (1994). Amantadine and memantine are NMDA receptor antagonists with neuroprotective properties. *Journal of Neural Transmission.Supplementum, 43*, 91-104.

Kosaka, K., Yoshimura, M., Ikeda, K., & Budka, H. (1984). Diffuse type of lewy body disease: Progressive dementia with abundant cortical lewy bodies and senile changes of varying degree--a new disease? *Clinical Neuropathology, 3*(5), 185-192.

Kosik, K. S., Joachim, C. L., & Selkoe, D. J. (1986). Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America, 83*(11), 4044-4048. doi:10.1073/pnas.83.11.4044 [doi]

Kovacs, G. G., Wagner, U., Dumont, B., Pikkarainen, M., Osman, A. A., Streichenberger, N., . . . Lachmann, I. (2012). An antibody with high reactivity for disease-associated alpha-synuclein reveals extensive brain pathology. *Acta Neuropathologica, 124*(1), 37-50. doi:10.1007/s00401-012-0964-x [doi]

Krasemann, S., Madore, C., Cialic, R., Baufeld, C., Calcagno, N., El Fatimy, R., . . . Butovsky, O. (2017). The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity, 47*(3), 566-581.e9. doi:S1074-7613(17)30366-7 [pii]

Kumar, V. (2019). A STING to inflammation and autoimmunity. *Journal of Leukocyte Biology, 106*(1), 171-185. doi:10.1002/JLB.4MIR1018-397RR [doi]

Kunkle, B. W., Grenier-Boley, B., Sims, R., Bis, J. C., Damotte, V., Naj, A. C., . . . Genetic and Environmental Risk in AD/Defining Genetic, Polygenic and Environmental Risk for Alzheimer's Disease Consortium (GERAD/PERADES). (2019). Genetic meta-analysis of diagnosed alzheimer's disease identifies new risk loci and implicates abeta, tau, immunity

and lipid processing. *Nature Genetics, 51*(3), 414-430. doi:10.1038/s41588-019-0358-2 [doi]

Kun-Rodrigues, C., Orme, T., Carmona, S., Hernandez, D. G., Ross, O. A., Eicher, J. D., . . . Bras, J. (2019). A comprehensive screening of copy number variability in dementia with lewy bodies. *Neurobiology of Aging, 75*, 223.e1-223.e10. doi:S0197-4580(18)30383-X [pii]

Laaksovirta, H., Peuralinna, T., Schymick, J. C., Scholz, S. W., Lai, S. L., Myllykangas, L., . . . Traynor, B. J. (2010). Chromosome 9p21 in amyotrophic lateral sclerosis in finland: A genome-wide association study. *The Lancet.Neurology, 9*(10), 978-985. doi:10.1016/S1474-4422(10)70184-8 [doi]

Lappalainen, T., Scott, A. J., Brandt, M., & Hall, I. M. (2019). Genomic analysis in the age of human genome sequencing. *Cell, 177*(1), 70-84. doi:S0092-8674(19)30215-6 [pii]

Lee, H. J., Lee, K., & Im, H. (2012). Alpha-synuclein modulates neurite outgrowth by interacting with SPTBN1. *Biochemical and Biophysical Research Communications, 424*(3), 497-502. doi:10.1016/j.bbrc.2012.06.143 [doi]

Lee, H., Zhang, Z., & Krause, H. M. (2019). Long noncoding RNAs and repetitive elements: Junk or intimate evolutionary partners? *Trends in Genetics : TIG, 35*(12), 892-902. doi:S0168-9525(19)30193-3 [pii]

Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., . . . Exome Aggregation Consortium. (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature, 536*(7616), 285-291. doi:10.1038/nature19057 [doi]

Leverenz, J. B., Umar, I., Wang, Q., Montine, T. J., McMillan, P. J., Tsuang, D. W., . . . Zhang, J. (2007). Proteomic identification of novel proteins in cortical lewy bodies. *Brain Pathology (Zurich, Switzerland), 17*(2), 139-145. doi:BPA048 [pii]

Levy-Lahad, E., Wijsman, E. M., Nemens, E., Anderson, L., Goddard, K. A., Weber, J. L., . . . Schellenberg, G. D. (1995). A familial alzheimer's disease locus on chromosome 1. *Science (New York, N.Y.), 269*(5226), 970-973. doi:10.1126/science.7638621 [doi]

Liu, J., & Wang, F. (2017). Role of neuroinflammation in amyotrophic lateral sclerosis: Cellular mechanisms and therapeutic implications. *Frontiers in Immunology, 8*, 1005. doi:10.3389/fimmu.2017.01005 [doi]

Liu, Y., Yu, J. T., Zong, Y., Zhou, J., & Tan, L. (2014). C9ORF72 mutations in neurodegenerative diseases. *Molecular Neurobiology, 49*(1), 386-398. doi:10.1007/s12035-013-8528-1 [doi]

Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell, 153*(6), 1194-1217. doi:10.1016/j.cell.2013.05.039 [doi]

Lynch, H. T., & de la Chapelle, A. (1999). Genetic susceptibility to non-polyposis colorectal cancer. *Journal of Medical Genetics, 36*(11), 801-818.

Majounie, E., Abramzon, Y., Renton, A. E., Perry, R., Bassett, S. S., Pletnikova, O., . . . Traynor, B. J. (2012). Repeat expansion in C9ORF72 in alzheimer's disease. *The New England Journal of Medicine, 366*(3), 283-284. doi:10.1056/NEJMc1113592 [doi]

Majounie, E., Renton, A. E., Mok, K., Dopper, E. G., Waite, A., Rollinson, S., . . . Traynor, B. J. (2012). Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: A cross-sectional study. *The Lancet.Neurology, 11*(4), 323-330. doi:10.1016/S1474-4422(12)70043-1 [doi]

Majounie, E., Abramzon, Y., Renton, A. E., Perry, R., Bassett, S. S., Pletnikova, O., . . . Traynor, B. J. (2012). Repeat expansion in C9ORF72 in alzheimer's disease. *The New England Journal of Medicine, 366*(3), 283-284. doi:10.1056/NEJMc1113592 [doi]

Mandybur, T. I. (1975). The incidence of cerebral amyloid angiopathy in alzheimer's disease. *Neurology, 25*(2), 120-126. doi:10.1212/wnl.25.2.120 [doi]

Marangi, G., & Traynor, B. J. (2015). Genetic causes of amyotrophic lateral sclerosis: New genetic analysis methodologies entailing new opportunities and challenges. *Brain Research, 1607*, 75-93. doi:10.1016/j.brainres.2014.10.009 [doi]

Masliah, E., Rockenstein, E., Veinbergs, I., Sagara, Y., Mallory, M., Hashimoto, M., & Mucke, L. (2001). Beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking alzheimer's disease and parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 98*(21), 12245-12250. doi:10.1073/pnas.211412398 [doi]

Mathieson, I., & McVean, G. (2012). Differential confounding of rare and common variants in spatially structured populations. *Nature Genetics, 44*(3), 243-246. doi:10.1038/ng.1074 [doi]

Maurano, M. T., Humbert, R., Rynes, E., Thurman, R. E., Haugen, E., Wang, H., . . . Stamatoyannopoulos, J. A. (2012). Systematic localization of common disease-associated variation in regulatory DNA. *Science (New York, N.Y.), 337*(6099), 1190-1195. doi:10.1126/science.1222794 [doi]

Maurel, C., Dangoumau, A., Marouillat, S., Brulard, C., Chami, A., Hergesheimer, R., . . . Vourc'h, P. (2018). Causative genes in amyotrophic lateral sclerosis and protein degradation pathways: A link to neurodegeneration. *Molecular Neurobiology, 55*(8), 6480-6499. doi:10.1007/s12035-017-0856-0 [doi]

McCauley, M. E., O'Rourke, J. G., Yanez, A., Markman, J. L., Ho, R., Wang, X., . . . Baloh, R. H. (2020). C9orf72 in myeloid cells suppresses STING-induced inflammation. *Nature,* doi:10.1038/s41586-020-2625-x [doi]

McComas, A. J., Sica, R. E., & Toyonaga, K. (1978). Incidence, severity, and time-course of motoneurone dysfunction in myotonic dystrophy: Their significance for an understanding of anticipation. *Journal of Neurology, Neurosurgery, and Psychiatry, 41*(10), 882-893. doi:10.1136/jnnp.41.10.882 [doi]

McKeith, I. G., Galasko, D., Kosaka, K., Perry, E. K., Dickson, D. W., Hansen, L. A., . . . Perry, R. H. (1996). Consensus guidelines for the clinical and pathologic diagnosis of dementia with lewy bodies (DLB): Report of the consortium on DLB international workshop. *Neurology, 47*(5), 1113-1124. doi:10.1212/wnl.47.5.1113 [doi]

McKeith, I. G., Boeve, B. F., Dickson, D. W., Halliday, G., Taylor, J. P., Weintraub, D., . . . Kosaka, K. (2017). Diagnosis and management of dementia with lewy bodies: Fourth

consensus report of the DLB consortium. *Neurology, 89*(1), 88-100. doi:10.1212/WNL.0000000000004058 [doi]

McKeith, I., O'Brien, J., Walker, Z., Tatsch, K., Booij, J., Darcourt, J., . . . DLB Study Group. (2007). Sensitivity and specificity of dopamine transporter imaging with 123I-FP-CIT SPECT in dementia with lewy bodies: A phase III, multicentre study. *The Lancet.Neurology, 6*(4), 305-313. doi:S1474-4422(07)70057-1 [pii]

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., . . . DePristo, M. A. (2010). The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research, 20*(9), 1297-1303. doi:10.1101/gr.107524.110 [doi]

McKhann, G. M., Knopman, D. S., Chertkow, H., Hyman, B. T., Jack, C. R., Kawas, C. H., . . . Phelps, C. H. (2011). The diagnosis of dementia due to alzheimer's disease: Recommendations from the national institute on aging-alzheimer's association workgroups on diagnostic guidelines for alzheimer's disease. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association, 7*(3), 263-269. doi:10.1016/j.jalz.2011.03.005 [doi]

Meeus, B., Nuytemans, K., Crosiers, D., Engelborghs, S., Peeters, K., Mattheijssens, M., . . . Theuns, J. (2010). Comprehensive genetic and mutation analysis of familial dementia with lewy bodies linked to 2q35-q36. *Journal of Alzheimer's Disease : JAD, 20*(1), 197-205. doi:10.3233/JAD-2010-1356 [doi]

Meinila, M., Finnila, S., & Majamaa, K. (2001). Evidence for mtDNA admixture between the finns and the saami. *Human Heredity, 52*(3), 160-170. doi:53372 [pii]

Meyer, K., Ferraiuolo, L., Miranda, C. J., Likhite, S., McElroy, S., Renusch, S., . . . Kaspar, B. K. (2014). Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS. *Proceedings of the National Academy of Sciences of the United States of America, 111*(2), 829-832. doi:10.1073/pnas.1314085111 [doi]

Miller, R. G., Mitchell, J. D., & Moore, D. H. (2012). Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *The Cochrane Database of Systematic Reviews, (3):CD001447. doi*(3), CD001447. doi:10.1002/14651858.CD001447.pub3 [doi]

Mirkin, S. M. (2007). Expandable DNA repeats and human disease. *Nature, 447*(7147), 932-940. doi:nature05977 [pii]

Mirra, S. S., Heyman, A., McKeel, D., Sumi, S. M., Crain, B. J., Brownlee, L. M., . . . Berg, L. (1991a). The consortium to establish a registry for alzheimer's disease (CERAD). part II. standardization of the neuropathologic assessment of alzheimer's disease. *Neurology, 41*(4), 479-486. doi:10.1212/wnl.41.4.479 [doi]

Mirra, S. S., Heyman, A., McKeel, D., Sumi, S. M., Crain, B. J., Brownlee, L. M., . . . Berg, L. (1991b). The consortium to establish a registry for alzheimer's disease (CERAD). part II. standardization of the neuropathologic assessment of alzheimer's disease. *Neurology, 41*(4), 479-486.

Mitt, M., Kals, M., Parn, K., Gabriel, S. B., Lander, E. S., Palotie, A., . . . Palta, P. (2017). Improved imputation accuracy of rare and low-frequency variants using population-specific high-coverage WGS-based imputation reference panel. *European Journal of Human Genetics : EJHG, 25*(7), 869-876. doi:10.1038/ejhg.2017.51 [doi]

Morrison, J. H., & Hof, P. R. (1997). Life and death of neurons in the aging brain. *Science (New York, N.Y.), 278*(5337), 412-419. doi:10.1126/science.278.5337.412 [doi]

Munch, C., O'Brien, J., & Bertolotti, A. (2011). Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. *Proceedings of the National Academy of Sciences of the United States of America, 108*(9), 3548-3553. doi:10.1073/pnas.1017275108 [doi]

Murros, K., & Fogelholm, R. (1983). Amyotrophic lateral sclerosis in middle-finland: An epidemiological study. *Acta Neurologica Scandinavica, 67*(1), 41-47. doi:10.1111/j.1600-0404.1983.tb04543.x [doi]

Myllykangas, L., Polvikoski, T., Reunanen, K., Wavrant-De Vrieze, F., Ellis, C., Hernandez, D., . . . Tienari, P. J. (2002). ApoE epsilon3-haplotype modulates alzheimer beta-amyloid deposition in the brain. *American Journal of Medical Genetics, 114*(3), 288-291. doi:10.1002/ajmg.10202 [pii]

Myllykangas, L., Polvikoski, T., Sulkava, R., Verkkoniemi, A., Crook, R., Tienari, P. J., . . . Perez-Tur, J. (1999). Genetic association of alpha2-macroglobulin with alzheimer's disease in a finnish elderly population. *Annals of Neurology, 46*(3), 382-390.

Nalls, M. A., Blauwendraat, C., Vallerga, C. L., Heilbron, K., Bandres-Ciga, S., Chang, D., . . . International Parkinson's Disease Genomics Consortium. (2019). Identification of novel risk loci, causal insights, and heritable risk for parkinson's disease: A meta-analysis of genome-wide association studies. *The Lancet.Neurology, 18*(12), 1091-1102. doi:S1474-4422(19)30320-5 [pii]

Nervi, A., Reitz, C., Tang, M. X., Santana, V., Piriz, A., Reyes, D., . . . Mayeux, R. (2011). Familial aggregation of dementia with lewy bodies. *Archives of Neurology, 68*(1), 90-93. doi:10.1001/archneurol.2010.319 [doi]

Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., . . . Lee, V. M. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science (New York, N.Y.), 314*(5796), 130-133. doi:314/5796/130 [pii]

Ng, A. S. L., & Tan, E. K. (2017). Intermediate C9orf72 alleles in neurological disorders: Does size really matter? *Journal of Medical Genetics, 54*(9), 591-597. doi:10.1136/jmedgenet-2017-104752 [doi]

Niemi, A. K., Hervonen, A., Hurme, M., Karhunen, P. J., Jylha, M., & Majamaa, K. (2003). Mitochondrial DNA polymorphisms associated with longevity in a finnish population. *Human Genetics, 112*(1), 29-33. doi:10.1007/s00439-002-0843-y [doi]

Nord, A. S., & West, A. E. (2019). Neurobiological functions of transcriptional enhancers. *Nature Neuroscience,* doi:10.1038/s41593-019-0538-5 [doi]

Norio, R. (2003a). Finnish disease heritage I: Characteristics, causes, background. *Human Genetics, 112*(5-6), 441-456. doi:10.1007/s00439-002-0875-3 [doi]

Norio, R. (2003b). Finnish disease heritage II: Population prehistory and genetic roots of finns. *Human Genetics, 112*(5-6), 457-469. doi:10.1007/s00439-002-0876-2 [doi]

Obi, T., Nishioka, K., Terada, T., Yamazaki, K., Sugiura, A., Takanashi, M., . . . Hattori, N. (2008). Clinicopathologic study of a SNCA gene duplication patient with parkinson disease and dementia. *Neurology, 70*(3), 238-241. doi:10.1212/01.wnl.0000299387.59159.db [doi]

Oinas, M., Polvikoski, T., Sulkava, R., Myllykangas, L., Juva, K., Notkola, I. L., . . . Paetau, A. (2009). Neuropathologic findings of dementia with lewy bodies (DLB) in a population-based vantaa 85+ study. *Journal of Alzheimer's Disease : JAD, 18*(3), 677-689. doi:10.3233/JAD-2009-1169 [doi]

Palo, J. U., Ulmanen, I., Lukka, M., Ellonen, P., & Sajantila, A. (2009). Genetic markers and population history: Finland revisited. *European Journal of Human Genetics : EJHG, 17*(10), 1336-1346. doi:10.1038/ejhg.2009.53 [doi]

Pantelakis, S. (1954). A particular type of senile angiopathy of the central nervous system: Congophilic angiopathy, topography and frequency. [Un type particuliar d'angiopathie senile du systeme nerveux central: l'angiopathie congophile; topographie et frequence] *Monatsschrift Fur Psychiatrie Und Neurologie, 128*(4), 219-256.

Petersen, R. C., Smith, G. E., Ivnik, R. J., Tangalos, E. G., Schaid, D. J., Thibodeau, S. N., . . . Kurland, L. T. (1995). Apolipoprotein E status as a predictor of the development of alzheimer's disease in memory-impaired individuals. *Jama, 273*(16), 1274-1278.

Peuralinna, T., Myllykangas, L., Oinas, M., Nalls, M. A., Keage, H. A., Isoviita, V. M., . . . Tienari, P. J. (2015). Genome-wide association study of neocortical lewy-related pathology. *Annals of Clinical and Translational Neurology, 2*(9), 920-931. doi:10.1002/acn3.231 [doi]

Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., . . . Hardiman, O. (2012). The syndrome of cognitive impairment in amyotrophic lateral sclerosis: A population-based study. *Journal of Neurology, Neurosurgery, and Psychiatry, 83*(1), 102-108. doi:10.1136/jnnp-2011-300188 [doi]

Piscopo, P., Marcon, G., Piras, M. R., Crestini, A., Campeggi, L. M., Deiana, E., . . . Confaloni, A. (2008). A novel PSEN2 mutation associated with a peculiar phenotype. *Neurology, 70*(17), 1549-1554. doi:10.1212/01.wnl.0000310643.53587.87 [doi]

Plaitakis, A., & Caroscio, J. T. (1987). Abnormal glutamate metabolism in amyotrophic lateral sclerosis. *Annals of Neurology, 22*(5), 575-579. doi:10.1002/ana.410220503 [doi]

Polymenidou, M., & Cleveland, D. W. (2011). The seeds of neurodegeneration: Prion-like spreading in ALS. *Cell, 147*(3), 498-508. doi:10.1016/j.cell.2011.10.011 [doi]

Price, J. L., Davis, P. B., Morris, J. C., & White, D. L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and alzheimer's disease. *Neurobiology of Aging, 12*(4), 295-312. doi:0197-4580(91)90006-6 [pii]

Price, J. L., & Morris, J. C. (1999). Tangles and plaques in nondemented aging and "preclinical" alzheimer's disease. *Annals of Neurology, 45*(3), 358-368. doi:10.1002/1531-8249(199903)45:33.0.co;2-x [doi]

Prokopenko, I., Miyakawa, G., Zheng, B., Heikkinen, J., Petrova Quayle, D., Udeh-Momoh, C., . . . Middleton, L. T. (2019). Alzheimer's disease pathology explains association between dementia with lewy bodies and APOE-epsilon4/TOMM40 long poly-T repeat allele variants. *Alzheimer's & Dementia (New York, N.Y.), 5*, 814-824. doi:10.1016/j.trci.2019.08.005 [doi]

Przedborski, S., Vila, M., & Jackson-Lewis, V. (2003). Neurodegeneration: What is it and where are we? *The Journal of Clinical Investigation, 111*(1), 3-10. doi:10.1172/JCI17522 [doi]

Rahkonen, T., Eloniemi-Sulkava, U., Rissanen, S., Vatanen, A., Viramo, P., & Sulkava, R. (2003). Dementia with lewy bodies according to the consensus criteria in a general population aged 75 years or older. *Journal of Neurology, Neurosurgery, and Psychiatry, 74*(6), 720-724.

Rajan, K. B., Wilson, R. S., Weuve, J., Barnes, L. L., & Evans, D. A. (2015). Cognitive impairment 18 years before clinical diagnosis of alzheimer disease dementia. *Neurology, 85*(10), 898-904. doi:10.1212/WNL.0000000000001774 [doi]

Rantalainen, V., Lahti, J., Henriksson, M., Kajantie, E., Tienari, P., Eriksson, J. G., & Raikkonen, K. (2016). APOE and aging-related cognitive change in a longitudinal cohort of men. *Neurobiology of Aging, 44*, 151-158. doi:S0197-4580(16)30061-6 [pii]

Renton, A. E., Chio, A., & Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nature Neuroscience, 17*(1), 17-23. doi:10.1038/nn.3584 [doi]

Renton, A. E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J. R., . . . Traynor, B. J. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron, 72*(2), 257-268. doi:10.1016/j.neuron.2011.09.010 [doi]

Rezania, K., Yan, J., Dellefave, L., Deng, H. X., Siddique, N., Pascuzzi, R. T., . . . Roos, R. P. (2003). A rare cu/zn superoxide dismutase mutation causing familial amyotrophic lateral sclerosis with variable age of onset, incomplete penetrance and a sensory neuropathy. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders : Official Publication of the World Federation of Neurology, Research Group on Motor Neuron Diseases, 4*(3), 162-166. doi:BUQ4E6Y0DMPHRW50 [pii]

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., . . . ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the american college of medical genetics and genomics and the association for molecular pathology. *Genetics in Medicine : Official Journal of the American College of Medical Genetics, 17*(5), 405-424. doi:10.1038/gim.2015.30 [doi]

Roberts, H. L., Schneider, B. L., & Brown, D. R. (2017). Alpha-synuclein increases beta-amyloid secretion by promoting beta-/gamma-secretase processing of APP. *PloS One, 12*(2), e0171925. doi:10.1371/journal.pone.0171925 [doi]

Robinson, J. L., Lee, E. B., Xie, S. X., Rennert, L., Suh, E., Bredenberg, C., . . . Trojanowski, J. Q. (2018). Neurodegenerative disease concomitant proteinopathies are prevalent, age-related and APOE4-associated. *Brain : A Journal of Neurology, 141*(7), 2181-2193. doi:10.1093/brain/awy146 [doi]

Rogers, S. L., & Friedhoff, L. T. (1996). The efficacy and safety of donepezil in patients with alzheimer's disease: Results of a US multicentre, randomized, double-blind, placebo-controlled trial. the donepezil study group. *Dementia (Basel, Switzerland), 7*(6), 293-303. doi:10.1159/000106895 [doi]

Rolfe, D. F., & Brown, G. C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological Reviews, 77*(3), 731-758. doi:10.1152/physrev.1997.77.3.731 [doi]

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., . . . Deng, H. X. (1993). Mutations in cu/zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature, 362*(6415), 59-62. doi:10.1038/362059a0 [doi]

Ruiz, A., Heilmann, S., Becker, T., Hernandez, I., Wagner, H., Thelen, M., . . . Ramirez, A. (2014). Follow-up of loci from the international genomics of alzheimer's disease project identifies TRIP4 as a novel susceptibility gene. *Translational Psychiatry, 4*, e358. doi:10.1038/tp.2014.2 [doi]

Schaffert, J., LoBue, C., White, C. L., Wilmoth, K., Didehbani, N., Lacritz, L., . . . Cullum, C. M. (2020). Risk factors for earlier dementia onset in autopsy-confirmed alzheimer's disease,

mixed alzheimer's with lewy bodies, and pure lewy body disease. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association,* doi:10.1002/alz.12049 [doi]

Schizophrenia Working Group of the Psychiatric Genomics Consortium. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature, 511*(7510), 421-427. doi:10.1038/nature13595 [doi]

Schneider, L. S., Dagerman, K. S., Higgins, J. P., & McShane, R. (2011). Lack of evidence for the efficacy of memantine in mild alzheimer disease. *Archives of Neurology, 68*(8), 991-998. doi:10.1001/archneurol.2011.69 [doi]

Selvaraj, B. T., Livesey, M. R., Zhao, C., Gregory, J. M., James, O. T., Cleary, E. M., . . . Chandran, S. (2018). C9ORF72 repeat expansion causes vulnerability of motor neurons to ca(2+)-permeable AMPA receptor-mediated excitotoxicity. *Nature Communications, 9*(1), 347-0. doi:10.1038/s41467-017-02729-0 [doi]

Seshadri, S., Fitzpatrick, A. L., Ikram, M. A., DeStefano, A. L., Gudnason, V., Boada, M., . . . EADI1 Consortium. (2010). Genome-wide analysis of genetic loci associated with alzheimer disease. *Jama, 303*(18), 1832-1840. doi:10.1001/jama.2010.574 [doi]

Shen, H., Li, J., Zhang, J., Xu, C., Jiang, Y., Wu, Z., . . . Deng, H. W. (2013). Comprehensive characterization of human genome variation by high coverage whole-genome sequencing of forty four caucasians. *PloS One, 8*(4), e59494. doi:10.1371/journal.pone.0059494 [doi]

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., . . . St George-Hyslop, P. H. (1995). Cloning of a gene bearing missense mutations in early-onset familial alzheimer's disease. *Nature, 375*(6534), 754-760. doi:10.1038/375754a0 [doi]

Singh, A., Kukreti, R., Saso, L., & Kukreti, S. (2019). Oxidative stress: A key modulator in neurodegenerative diseases. *Molecules (Basel, Switzerland), 24*(8), 10.3390/molecules24081583. doi:E1583 [pii]

Sperling, R. A., Aisen, P. S., Beckett, L. A., Bennett, D. A., Craft, S., Fagan, A. M., . . . Phelps, C. H. (2011). Toward defining the preclinical stages of alzheimer's disease: Recommendations from the national institute on aging-alzheimer's association workgroups on diagnostic guidelines for alzheimer's disease. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association, 7*(3), 280-292. doi:10.1016/j.jalz.2011.03.003 [doi]

Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S., & Roses, A. D. (1993). Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America, 90*(5), 1977-1981. doi:10.1073/pnas.90.5.1977 [doi]

Tarafdar, A., & Pula, G. (2018). The role of NADPH oxidases and oxidative stress in neurodegenerative disorders. *International Journal of Molecular Sciences, 19*(12), 10.3390/ijms19123824. doi:E3824 [pii]

Taylor, J. P., McKeith, I. G., Burn, D. J., Boeve, B. F., Weintraub, D., Bamford, C., . . . O'Brien, J. T. (2019). New evidence on the management of lewy body dementia. *The Lancet.Neurology,* doi:S1474-4422(19)30153-X [pii]

Thakur, P., Chiu, W. H., Roeper, J., & Goldberg, J. A. (2019). Alpha-synuclein 2.0 - moving towards cell type specific pathophysiology. *Neuroscience, 412*, 248-256. doi:S0306-4522(19)30404-X [pii]

Tsuang, D. W., Dalan, A. M., Eugenio, C. J., Poorkaj, P., Limprasert, P., La Spada, A. R., . . . Leverenz, J. B. (2002). Familial dementia with lewy bodies: A clinical and neuropathological study of 2 families. *Archives of Neurology, 59*(10), 1622-1630. doi:nob20039 [pii]

Tsuang, D., Leverenz, J. B., Lopez, O. L., Hamilton, R. L., Bennett, D. A., Schneider, J. A., . . . Zabetian, C. P. (2013). APOE epsilon4 increases risk for dementia in pure synucleinopathies. *JAMA Neurology, 70*(2), 223-228. doi:10.1001/jamaneurol.2013.600 [doi]

Turner, M. R., Goldacre, R., Ramagopalan, S., Talbot, K., & Goldacre, M. J. (2013). Autoimmune disease preceding amyotrophic lateral sclerosis: An epidemiologic study. *Neurology, 81*(14), 1222-1225. doi:10.1212/WNL.0b013e3182a6cc13 [doi]

Uchikado, H., Lin, W. L., DeLucia, M. W., & Dickson, D. W. (2006). Alzheimer disease with amygdala lewy bodies: A distinct form of alpha-synucleinopathy. *Journal of Neuropathology and Experimental Neurology, 65*(7), 685-697. doi:10.1097/01.jnen.0000225908.90052.07 [doi]

Uusvaara, J., Pitkala, K. H., Kautiainen, H., Tilvis, R. S., & Strandberg, T. E. (2013). Detailed cognitive function and use of drugs with anticholinergic properties in older people: A

community-based cross-sectional study. *Drugs & Aging, 30*(3), 177-182. doi:10.1007/s40266-013-0055-2 [doi]

van der Zee, J., Gijselinck, I., Dillen, L., Van Langenhove, T., Theuns, J., Engelborghs, S., . . . European Early-Onset Dementia Consortium. (2013). A pan-european study of the C9orf72 repeat associated with FTLD: Geographic prevalence, genomic instability, and intermediate repeats. *Human Mutation, 34*(2), 363-373. doi:10.1002/humu.22244 [doi]

Vann Jones, S. A., & O'Brien, J. T. (2014). The prevalence and incidence of dementia with lewy bodies: A systematic review of population and clinical studies. *Psychological Medicine, 44*(4), 673-683. doi:10.1017/S0033291713000494 [doi]

Varatharajah, Y., Ramanan, V. K., Iyer, R., Vemuri, P., & Alzheimer's Disease Neuroimaging Initiative. (2019). Predicting short-term MCI-to-AD progression using imaging, CSF, genetic factors, cognitive resilience, and demographics. *Scientific Reports, 9*(1), 2235-3. doi:10.1038/s41598-019-38793-3 [doi]

Visscher, P. M., Wray, N. R., Zhang, Q., Sklar, P., McCarthy, M. I., Brown, M. A., & Yang, J. (2017). 10 years of GWAS discovery: Biology, function, and translation. *American Journal of Human Genetics, 101*(1), 5-22. doi:S0002-9297(17)30240-9 [pii]

Wheeler, V. C., Persichetti, F., McNeil, S. M., Mysore, J. S., Mysore, S. S., MacDonald, M. E., . . . US-Venezuela Collaborative Research Group. (2007). Factors associated with HD CAG repeat instability in huntington disease. *Journal of Medical Genetics, 44*(11), 695-701. doi:jmg.2007.050930 [pii]

Witman, G. B., Cleveland, D. W., Weingarten, M. D., & Kirschner, M. W. (1976). Tubulin requires tau for growth onto microtubule initiating sites. *Proceedings of the National Academy of Sciences of the United States of America, 73*(11), 4070-4074. doi:10.1073/pnas.73.11.4070 [doi]

Wood, A. R., Esko, T., Yang, J., Vedantam, S., Pers, T. H., Gustafsson, S., . . . Frayling, T. M. (2014). Defining the role of common variation in the genomic and biological architecture of adult human height. *Nature Genetics, 46*(11), 1173-1186. doi:10.1038/ng.3097 [doi]

Yamada, M., Komatsu, J., Nakamura, K., Sakai, K., Samuraki-Yokohama, M., Nakajima, K., & Yoshita, M. (2019). Diagnostic criteria for dementia with lewy bodies: Updates and future directions. *Journal of Movement Disorders,* doi:10.14802/jmd.19052 [doi]

Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., . . . de Yebenes, J. G. (2004). The new mutation, E46K, of alpha-synuclein causes parkinson and lewy body dementia. *Annals of Neurology, 55*(2), 164-173. doi:10.1002/ana.10795 [doi]

Zhao, N., Attrebi, O. N., Ren, Y., Qiao, W., Sonustun, B., Martens, Y. A., . . . Bu, G. (2020). APOE4 exacerbates alpha-synuclein pathology and related toxicity independent of amyloid. *Science Translational Medicine, 12*(529), 10.1126/scitranslmed.aay1809. doi:eaay1809 [pii]